Agilent MassHunter Workstation Software

Quantitative Analysis

Familiarization Guide

Agilent Technologies

Notices

Manual Part Number

G3335-90061

Edition

Fourth Edition, April 2010

Printed in USA

Agilent Technologies, Inc.
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Santa Clara, CA USA 95051

Software Revision

This guide is valid for the B.04.xx or later revision of the Agilent MassHunter Workstation Software - Quantitative Analysis program, until superseded.

If you have comments about this guide, please send an e-mail to feedback_lcms@agilent.com.

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A WARNING notice denotes a hazard. It calls attention to an operating procedure, practice, or the like that, if not correctly performed or adhered to, could result in personal injury or death. Do not proceed beyond a WARNING notice until the indicated conditions are fully understood and met.

In this Guide...

The *Familiarization Guide* presents step-by-step exercises to help you learn to use the Quantitative Analysis program. You can do these exercises with the demo batch DrugsOfAbuse (Exercises 1 and 3 through 5) and Verapamil-targeted (Exercise 2), shipped with the system (in the **Data** folder of your installation disk), or with data you acquire.

The DrugsOfAbuse batch consists of MRM data files acquired on the Agilent 6410 Triple Quad LC/MS system. The Verapamil batch consists of Q-TOF data files acquired on the Agilent 6500 Series Q-TOF LC/MS system.

1 Set Up and Quantitate a Batch of Acquired MRM Data Files

In this exercise, you set up a batch table, a quantitation method, and target compounds, using acquired datafiles. Finally, you analyze the batch and save the results. This chapter is applicable for uses of the Agilent 6410 Triple Quad LC/MS system and the Agilent 7000A Triple Quad GC/MS system.

2 Set Up and Quantitate a Batch of Acquired Q-TOF Data Files

In this exercise, you set up a batch table, a quantitation method, and a target compound, using acquired datafiles. Finally, you analyze the batch and save the results.

3 Review Quantitation Results

In this exercise, you inspect the sample and compound data in a batch file, customize layouts, and export your batch results to a Microsoft Excel file.

4 Use Tools to Evaluate Results

The tools in this exercise make it easier for you to evaluate and obtain more accurate quantitation results.

5 Work With Quantitation Reports

In this exercise, you generate reports using specified templates, then review these reports in Microsoft Excel.

Choosing the Correct Quantitative Analysis Icon

You will find four different icons installed on the desktop when you install the Quantitative Analysis program. When you start the Quantitative Analysis program from these icons, the default values and some of the features are customized to the appropriate instrument type.

When you click the Quantitative Analysis icon on the desktop, the full name of the icon is displayed. Make sure you choose the icon that matches the type of data in the batch you want to analyze.

Before You Begin These Exercises

Copy the folder named Data from your installation disk in uncompressed format to any location on your hard disk.

This folder contains all of the data files needed for these exercises. You may need to first extract the data files from their zip format.

Do not reuse the example data files already on your system unless you know that you copied them from the originals on the disk and you are the only one using them. If the example data files already on the system do not match the original ones on the disk exactly, then the results obtained during these exercises will not match those shown in this guide.

Contents

Exercise 1
Set Up and Quantitate a Batch of Acquired MRM Data Files

In this exercise you set up a quantitation method for a batch of acquired data files. You carry out the exercise with the **DrugsOfAbuse** data files on your installation disk and learn how to perform the following tasks:

- Set up a Batch Table containing unknown sample and calibration data files for drugs of abuse: amphetamine, cocaine, methamphetamine, and MDMA.
- Set up a new quantitation method based on the calibration standard of the highest concentration.
- Set up target compounds.
  - View the MRM transitions and chromatographic parameters for the compounds in the data file.
  - Set up an internal standard for each of the compounds.
- Set up quantitation for the method.
  - Enter the concentration of the highest concentration calibration standard and the dilution pattern.
  - Set up qualifier ions and the calibration curve.
- Automatically quantitate the batch and save the results.

Each exercise is presented in a table with three columns:

- Steps – Use these general instructions to proceed on your own to explore the program.
- Detailed Instructions – Use these if you need help or prefer to use a step-by-step learning process.
- Comments – Read these to learn tips and additional information about each step in the exercise.

Before you begin...

Make sure that you have copied the **DrugsOfAbuse** folder from the **Data** folder of the installation disk to a folder on your system.

Task 1. Set Up a New Batch

In this task you set up a Batch Table containing data files for three unknown samples and several calibration samples of drugs of abuse: amphetamine, cocaine, methamphetamine and MDMA.

Steps	Detailed instructions	Comments
1 Create a new batch to hold samples. • Select all of the data files from the **DrugsOfAbuse** folder. • Name the batch file, ***iii*_test_01**, where the letters "*iii*" are your initials.	**a** To start the Quantitative Analysis program, click the **Quantitative Analysis (QQQ)** icon on your Desktop.  When you first use the program, the default layout appears, as shown in Figure 1.	• You can also access the program by clicking **Programs > Agilent > MassHunter Workstation > Quantitative Analysis (QQQ)** from the Start menu. • Different features are available when you are working with QQQ data.

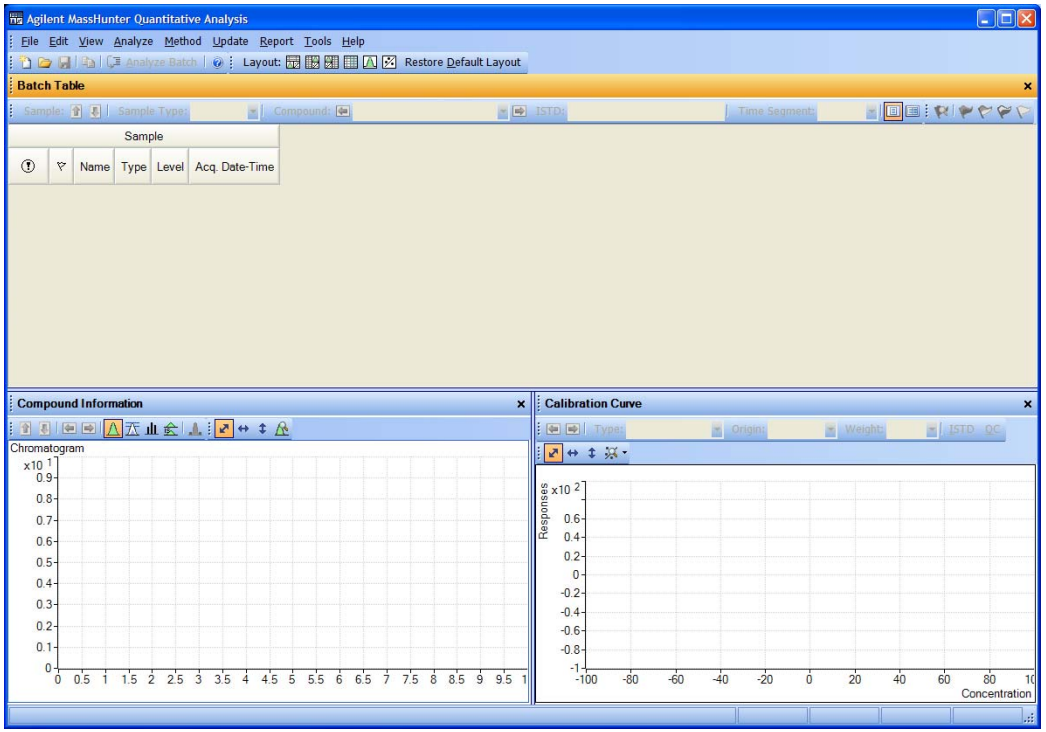
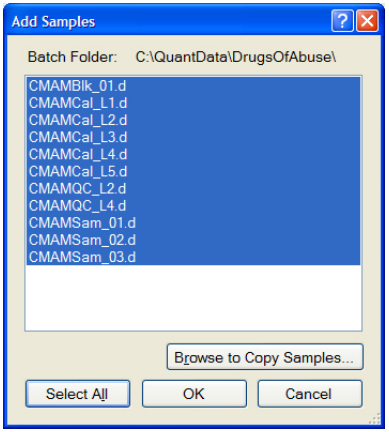


Figure 1 Default layout

Steps	Detailed instructions	Comments
	b Click **File > New Batch**. The system opens the **New Batch** dialog box. **c** Navigate to the folder ***Your Directory*\DrugsOfAbuse\.** **d** Type the batch filename ***iii*_Test_01** and click **Open**.	• If the default layout is not present, click **Restore Default Layout** on the toolbar before creating a new batch. Restore Default Layout
2 Add all the samples in the **DrugsOfAbuse** folder to the batch.	**a** Click **File > Add Samples**: The system displays the **Add Samples** dialog box. **b** Click **Select All** to select all samples, and then click **OK** to add them to the batch. The **Batch Table** is no longer empty. It now contains the calibration, QC, and unknown samples. See Figure 2. 	• Note that only three of the files are unknown samples, one is a blank five are calibration files at different calibration levels, and two are QC samples.

Steps	Detailed instructions	Comments

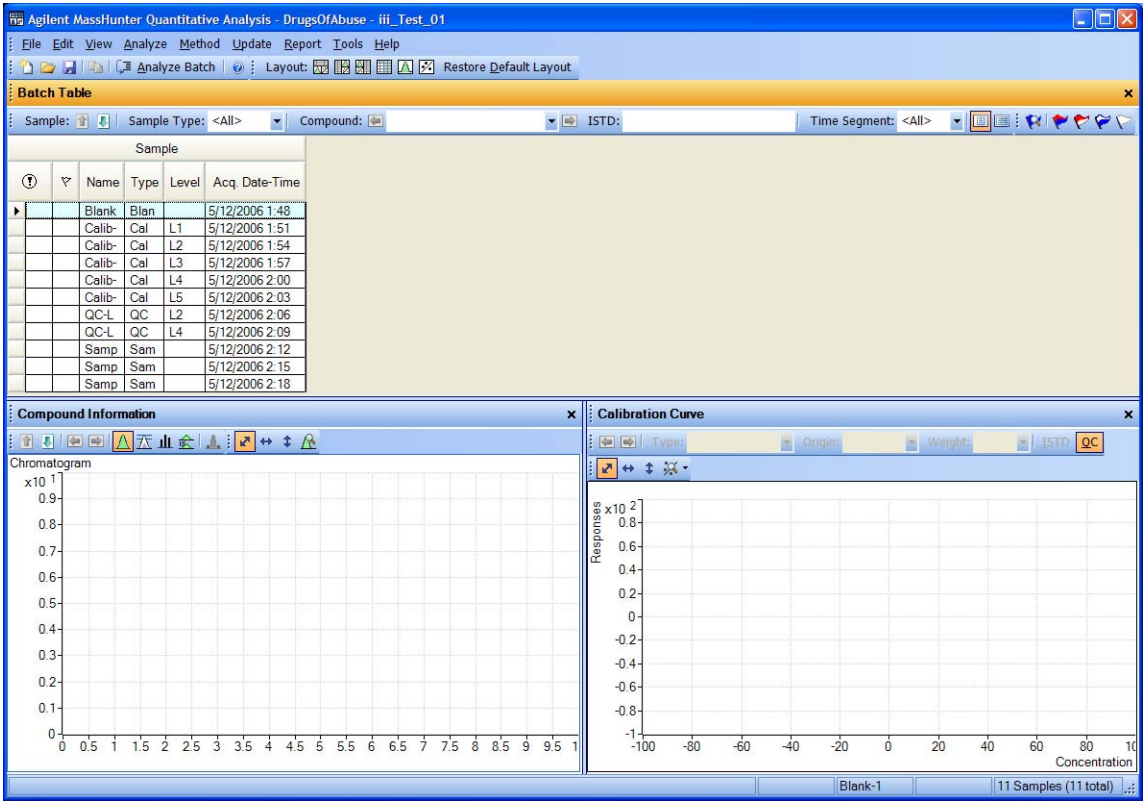
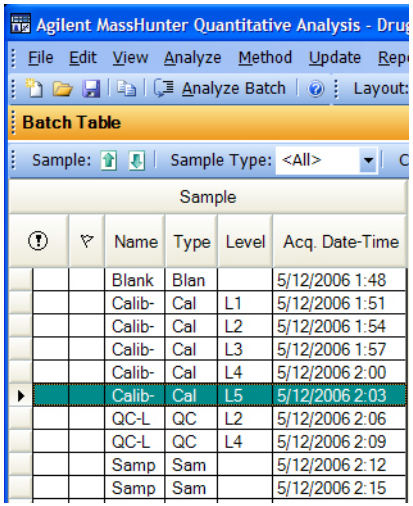


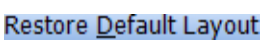
Figure 2 Batch Table containing Drugs of Abuse samples before quantitation

Task 2. Set Up a New Method for the Batch

This task shows you how to set up a new quantitation method based on the calibration data file with the highest concentration of sample.

Steps	Detailed Instructions	Comments
1 Create a new method from acquired MRM data. • Use the calibration data file with the highest signal.	**a** Use the cursor to highlight the calibration standard that has the highest concentration level, as shown in the figure below.	• Using a sample with strong signals for the compounds, such as a high-concentration calibration sample, lets the program create a method with the appropriate retention times and qualifier ratios.



	b Click **Method > Edit** to switch to method editing mode. The **Method Tasks** appear in the column to the left of the View, as shown in Figure 3.	• Note that Figure 3 shows the default layout for method editing. • If the default layout is not present, click **Restore Default Layout** on the toolbar before creating a new method in the next step. 

Steps	Detailed Instructions	Comments

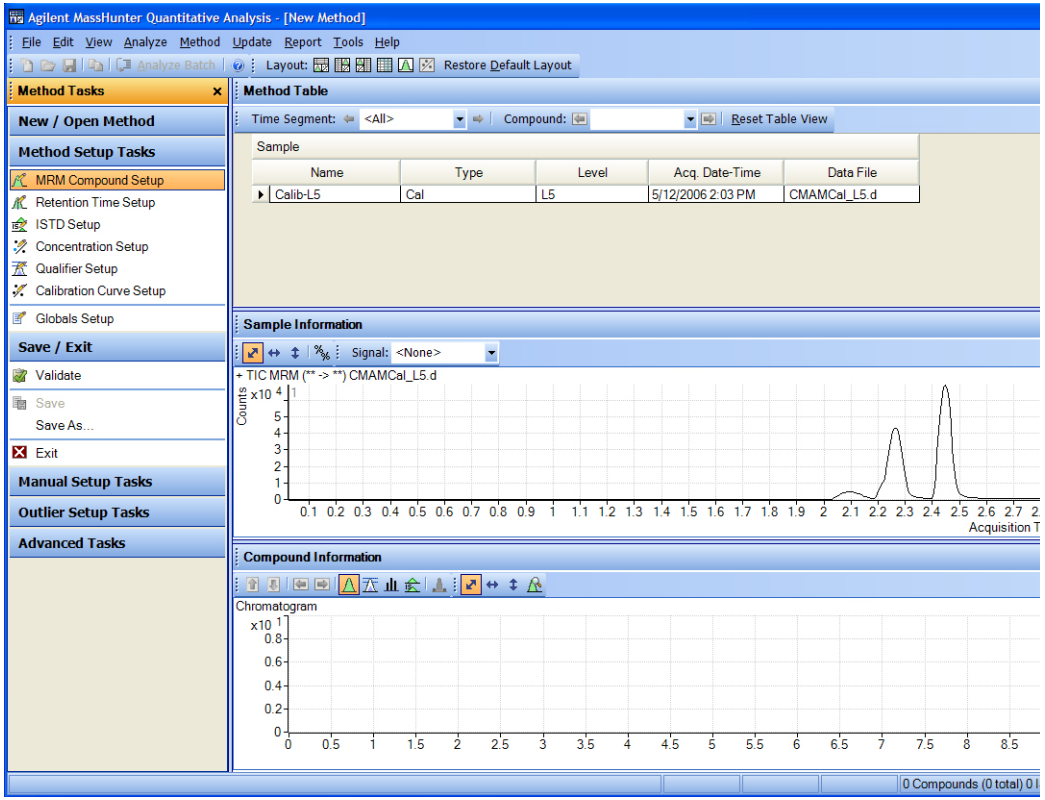
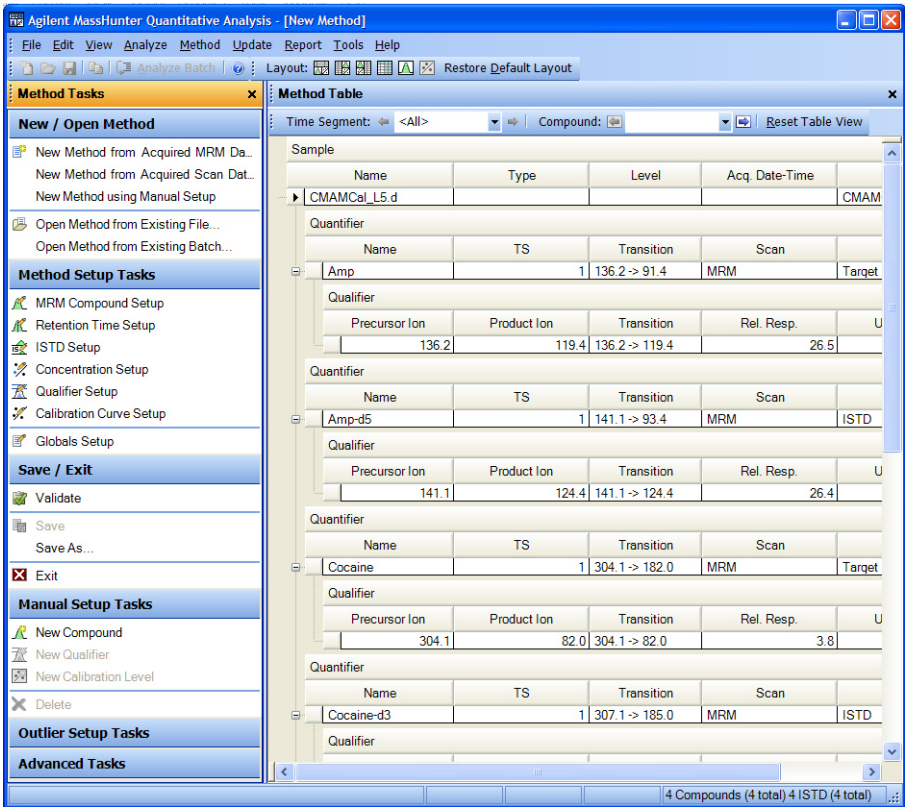


Figure 3 Method Edit mode

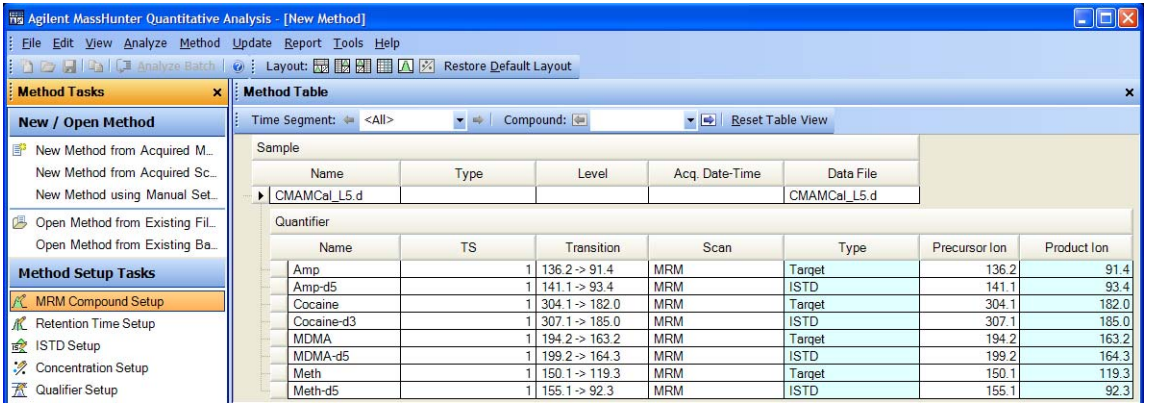
Steps	Detailed Instructions	Comments
	c Under **Method Tasks** in the sidebar to the left of the **Method Table**, click **New/Open Method > New Method from Acquired MRM Data**. The system displays a **Please select a sample folder...** dialog box. **d** Click **CMAMCal_L5.d** and click **Open** to import acquisition method information.	• You can also click **Method > New > New Method from Acquired MRM Data.** • The figure below shows the default layout for the level 5 calibration standard.



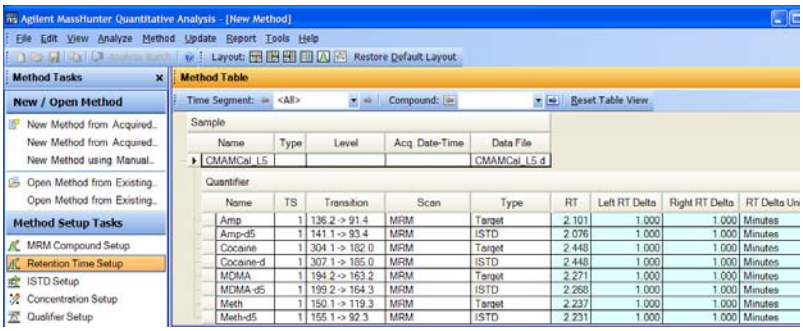
Task 3. Set Up Target Compounds

With this task you learn to inspect the MRM transitions and the RT data for the new quantitation method, which you can change for individual target compounds. You also learn to set up an ISTD compound for each target compound.

Steps	Detailed Instructions	Comments
1 Check the new quantitation method created from the imported acquisition method for MRM transitions.	**a** Under **Me2od Tasks** in the sidebar to the left of the **Method Table** window, click **Method Setup Tasks > MRM Compound Setup.**	• The compound names associated with MRM transitions are entered in the acquisition method. By default, the largest signal is chosen as the quantifier ion.



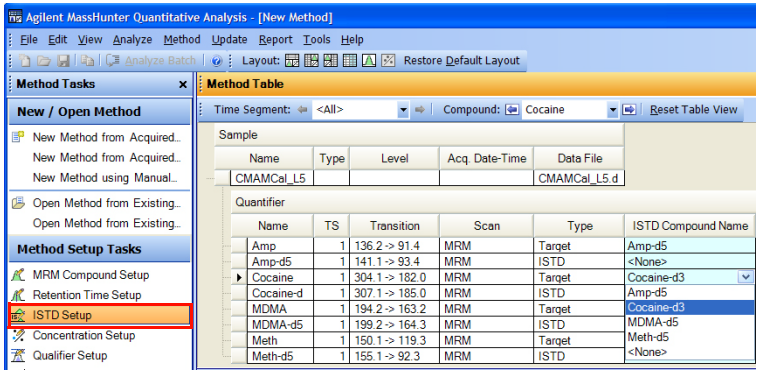
Steps	Detailed Instructions	Comments
	b To inspect the imported retention time data, click **Method Setup Tasks > Retention Time Setup**.	• You can modify data fields in blue for individual compounds.



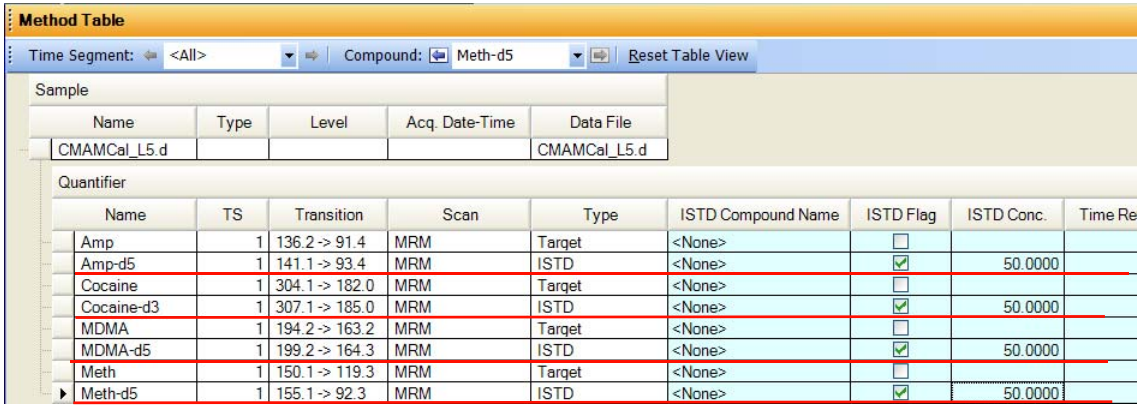
2 Set up ISTD compounds.
- Assign the corresponding deuterated compound as the internal standard (ISTD) for each target compound.

a Click **Method Setup Tasks > ISTD Setup**.
b For each target compound row, click the down arrow in the **ISTD Compound Name** cell.

- Do not attempt to enter the ISTD name into the ISTD compound row.



Steps	Detailed Instructions	Comments
	c Click the ISTD name associated with the target compound. **d** Type the ISTD concentration (**ISTD Conc.**) for each ISTD compound.	



Method Table

Time Segment: <All> Compound: Meth-d5 Reset Table View

Sample

Name	Type	Level	Acq. Date-Time	Data File
CMAMCal_L5.d				CMAMCal_L5.d

Quantifier

Name	TS	Transition	Scan	Type	ISTD Compound Name	ISTD Flag	ISTD Conc.	Time Re
Amp	1	136.2 -> 91.4	MRM	Target	<None>	☐		
Amp-d5	1	141.1 -> 93.4	MRM	ISTD	<None>	☑	50.0000	
Cocaine	1	304.1 -> 182.0	MRM	Target	<None>	☐		
Cocaine-d3	1	307.1 -> 185.0	MRM	ISTD	<None>	☑	50.0000	
MDMA	1	194.2 -> 163.2	MRM	Target	<None>	☐		
MDMA-d5	1	199.2 -> 164.3	MRM	ISTD	<None>	☑	50.0000	
Meth	1	150.1 -> 119.3	MRM	Target	<None>	☐		
Meth-d5	1	155.1 -> 92.3	MRM	ISTD	<None>	☑	50.0000	

Task 4. Set Up Quantitation

This task presents instructions for setting up the quantitation parameters for the method's:

- Calibration levels
- Qualifier ions
- Calibration curve fit

Steps	Detailed instructions	Comments
1 Create five calibration levels for each compound. • Set the highest concentration for amphetamine at 125. • Set a dilution pattern of 1:5:2:2.5:2 for amphetamine. • Compare the concentrations for the five levels with the dilution pattern.	**a** Click **Method Setup Tasks > Concentration Setup**, and type `125` in the **Dil. High Conc**. column for amphetamine (Amp). **b** Type `1:5:2:2.5:2` in the **Dil. Pattern** column for Amp. **c** Make sure **Level Name Prefix** is **L** and **# of Levels** is **5** in the **Serial Dilution** toolbar.	

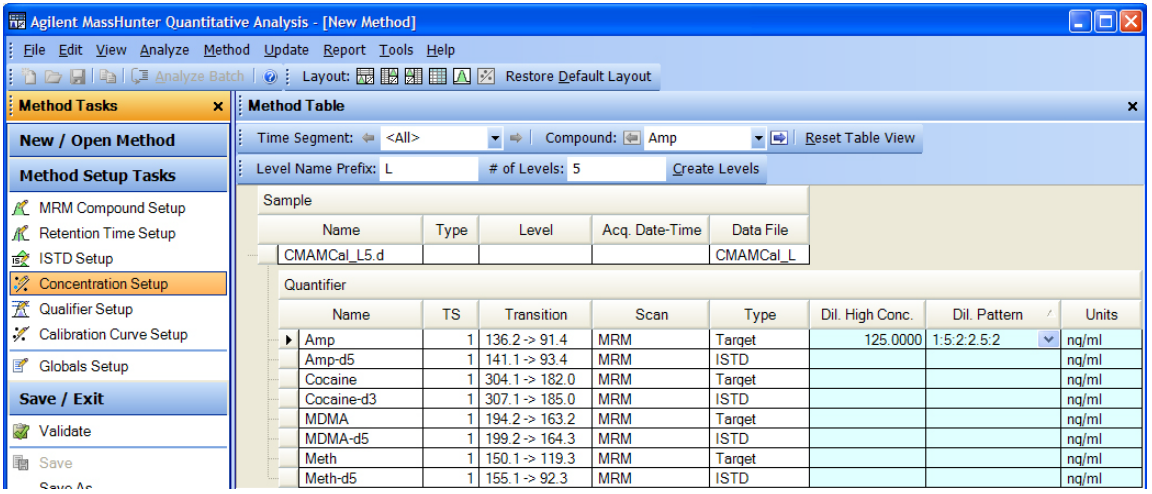
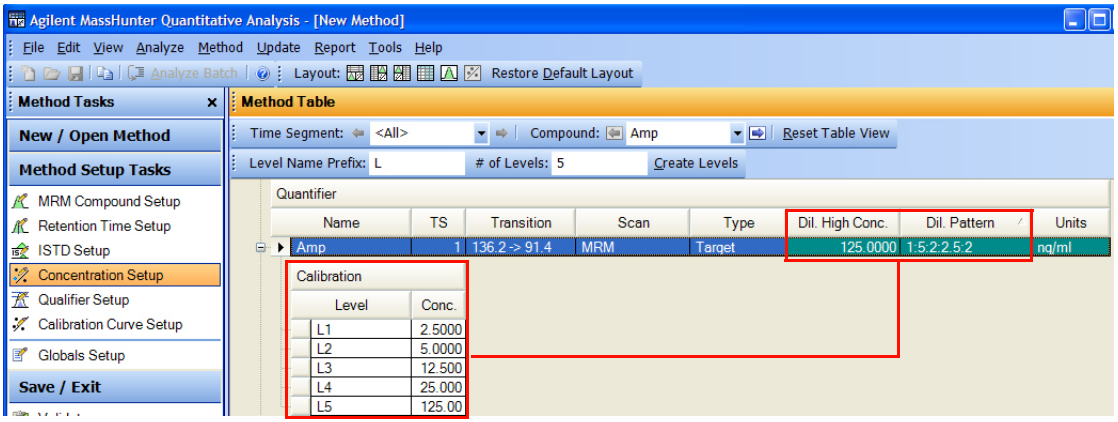


Figure 4 Creating five calibration levels for first compound

Steps	Detailed instructions	Comments
	d Click **Create Levels**. **e** Compare the newly created calibration levels with Dilution High Concentration and Dilution Pattern.	• After you create the calibration table for amphetamine, you tell the program to copy this table to the other target compounds in step 2.

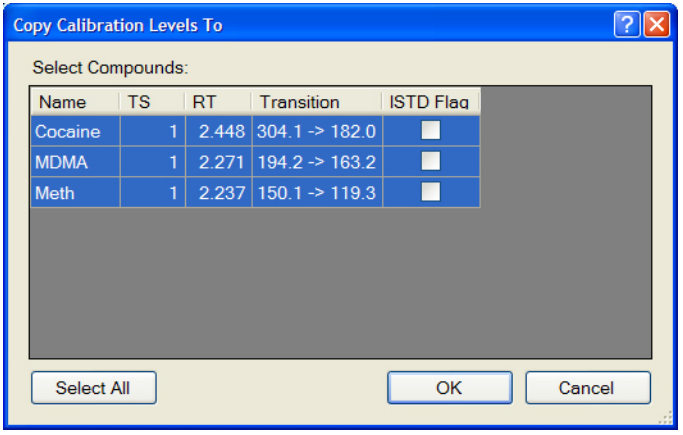


2 Copy the calibration levels and concentrations to the other compounds.

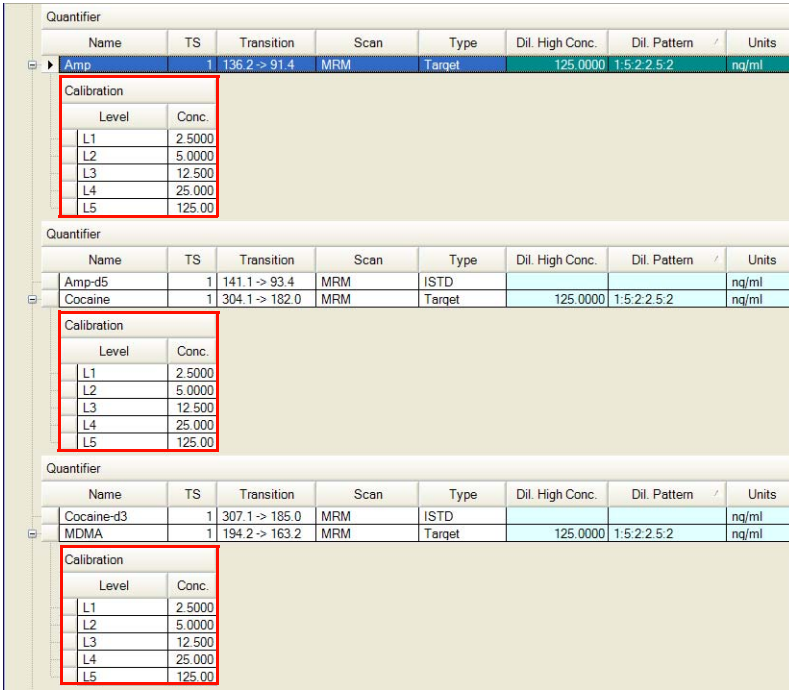
- Close the **Compound Information** window.
- Compare the calibration setup for the four compounds.

a Click **Method > Copy Calibration Levels To...**
The system displays the **Copy Calibration Levels To** dialog box.

b Click **Select All**, and then click **OK**.



Steps	Detailed instructions	Comments
	c Close the **Compound Information** window and the **Sample Information** window in the lower half of the Quantitative Data Analysis main view. **d** Browse the **Method Table** to compare the calibration concentration setup among the four target compounds, Amp, Cocaine, Meth, and MDMA	



Quantifier

Name	TS	Transition	Scan	Type	Dil. High Conc.	Dil. Pattern	Units
Amp	1	136.2 -> 91.4	MRM	Target	125.0000	1:5:2:2.5:2	ng/ml

Calibration

Level	Conc.
L1	2.5000
L2	5.0000
L3	12.500
L4	25.000
L5	125.00

Quantifier

Name	TS	Transition	Scan	Type	Dil. High Conc.	Dil. Pattern	Units
Amp-d5	1	141.1 -> 93.4	MRM	ISTD			ng/ml
Cocaine	1	304.1 -> 182.0	MRM	Target	125.0000	1:5:2:2.5:2	ng/ml

Calibration

Level	Conc.
L1	2.5000
L2	5.0000
L3	12.500
L4	25.000
L5	125.00

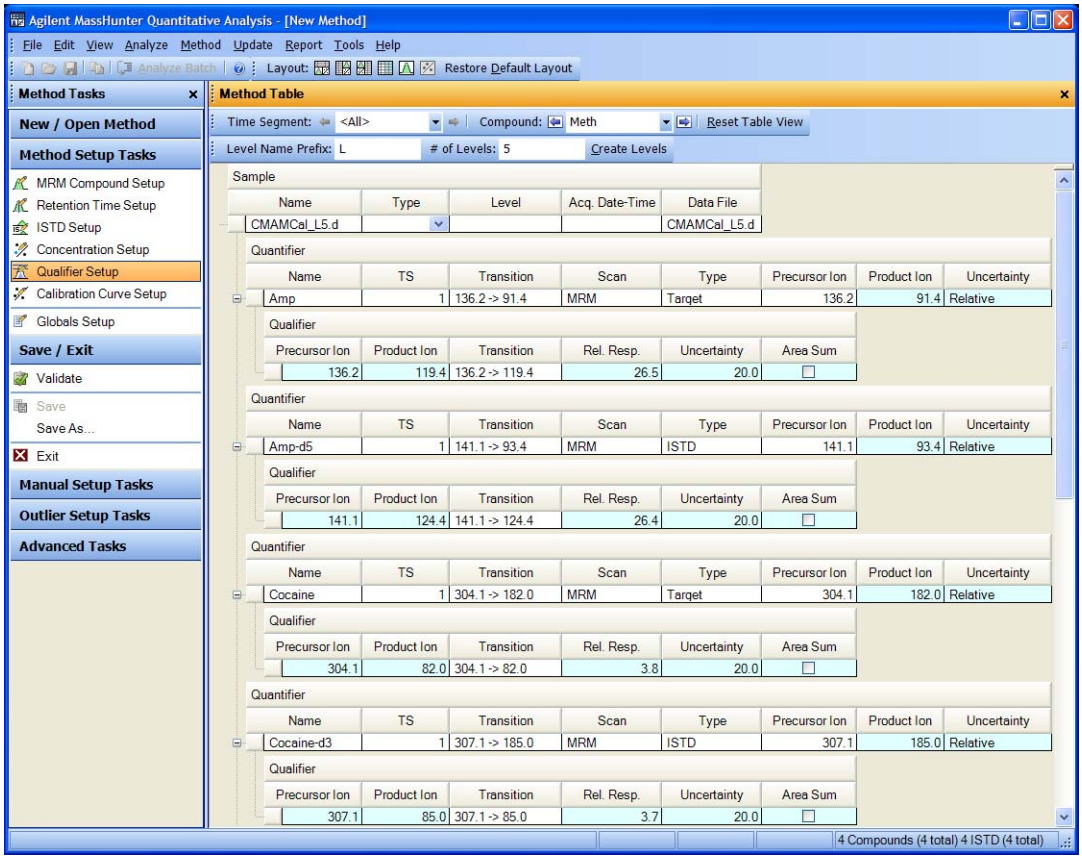
Quantifier

Name	TS	Transition	Scan	Type	Dil. High Conc.	Dil. Pattern	Units
Cocaine-d3	1	307.1 -> 185.0	MRM	ISTD			ng/ml
MDMA	1	194.2 -> 163.2	MRM	Target	125.0000	1:5:2:2.5:2	ng/ml

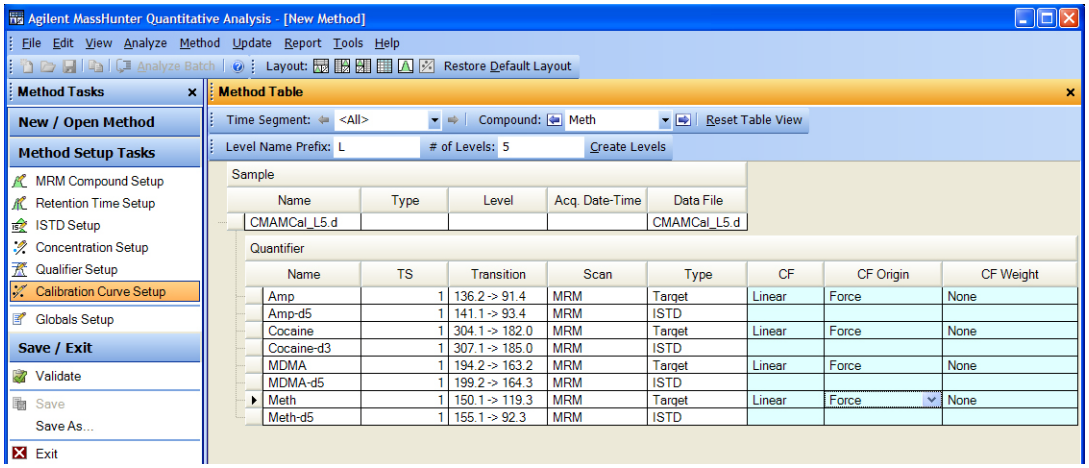
Calibration

Level	Conc.
L1	2.5000
L2	5.0000
L3	12.500
L4	25.000
L5	125.00

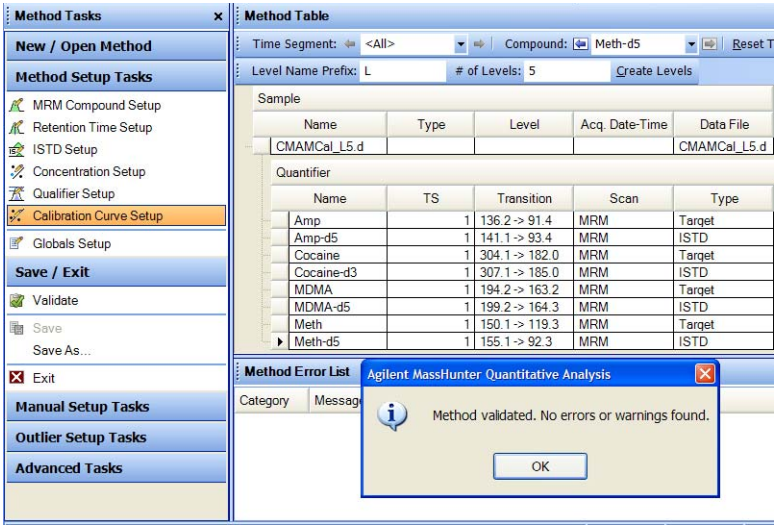
Steps	Detailed instructions	Comments
3 Set up qualifier ions and a calibration curve. • Review the Qualifier setup parameters. • Change the default curve origin from Linear to Force.	**a** Click **Method Tasks > Qualifier Setup**, and inspect the Qualifier setup parameters.	• The system automatically populates the qualifier setup parameters when it imports MRM acquisition information. • During method creation, additional MRM transitions besides the quantifier ion for a compound are assigned as qualifier ions.



Steps	Detailed instructions	Comments
	b Click **Method Tasks > Calibration Curve Setup**. **c** For each target compound change the **CF Origin** to **Force**.	



Steps	Detailed instructions	Comments
4 Validate and save the method.	**a** Click **Save/Exit > Validate** to validate the method setup.	• You can view any validation errors that do occur at the bottom of the screen.



b After the validation message appears, click **OK.**

c Click **Save/Exit > Exit**, and click **Yes** to the **Would you like to apply this method to the batch?** prompt.

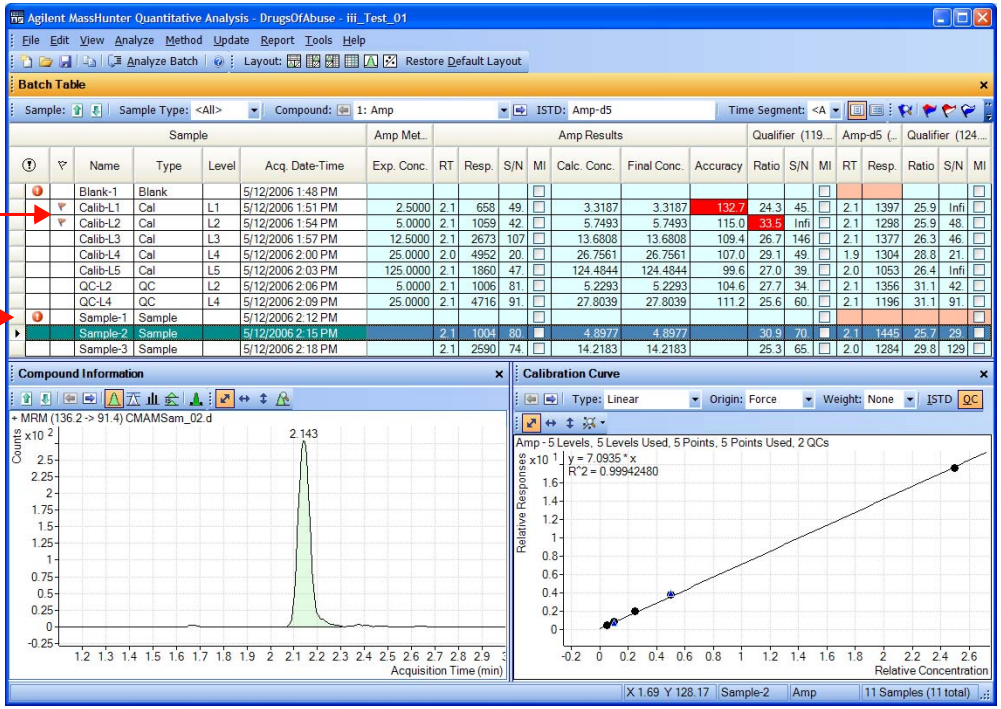
Task 5. Analyze and Save the Batch

In this exercise you automatically quantitate the batch and then save the results.

Steps	Detailed instructions	Comments
1 Analyze the batch, and inspect the results for each compound. • Examine the Quantitation Message(s), which identify samples with no quantitated signals. • Examine the outlier flag messages.	**a** Click the **Analyze Batch** icon (Analyze Batch) in the toolbar to start batch analysis. **b** Pass the cursor over the quantitation message for Sample 1. **c** Pass the cursor over the flags for the first two calibration standards.	• Note that the program found no data for Amphetamine (Amp) in Sample-1. • Note that two calibration standards contain outlier data.

Outlier flag messages

Quantitation message



2 Save the batch.

a Click **File > Save Batch**.
b Click **File > Close Batch** to close the batch.

Exercise 2
Set Up and Quantitate a Batch of Acquired Q-TOF Data Files

In this exercise you set up a quantitation method for a batch of acquired Q-TOF data files. You carry out the exercise with the **Verapamil** data files on your installation disk and learn how to perform the following tasks:

- Set up a Batch Table containing blank and calibration data files for verapamil.
- Set up a new quantitation method based on the calibration standard of the highest concentration.
- Set up a target compound.
  - View the product ion and chromatographic parameters for the verapamil compound in the data file.
- Set up quantitation for the method.
  - Enter the concentration of the highest concentration calibration standard and the dilution pattern.
  - Set up qualifier ions and the calibration curve.
- Automatically quantitate the batch and save the results.

Each exercise is presented in a table with three columns:

- Steps – Use these general instructions to proceed on your own to explore the program.
- Detailed Instructions – Use these if you need help or prefer to use a step-by-step learning process.
- Comments – Read these to learn tips and additional information about each step in the exercise.

Before you begin...

Make sure that you have copied the **Verapamil-targetedMSMS** folder from the **Data/QTOF** folder of the installation disk to a folder on your system.

Task 1. Set Up a New Batch

In this task you set up a Batch Table containing data files for calibration samples of verapamil. Many of the tasks in this section are similar to the tasks in Exercise 1.

Steps	Detailed instructions	Comments
1 Create a new batch to hold samples. • Select all of the data files from the **Verapamil-targetedMSMS** folder. • Name the batch file, ***iii*_test_01**, where "*iii*" are your initials.	**a** To start the Quantitative Analysis program, click the **Quantitative Analysis (Q-TOF)** icon on your Desktop. When you first use the program, the default layout appears, as shown in Figure 5.	• You can also access the program by clicking **Programs > Agilent > MassHunter Workstation > Quantitative Analysis (Q-TOF)** from the Start menu.

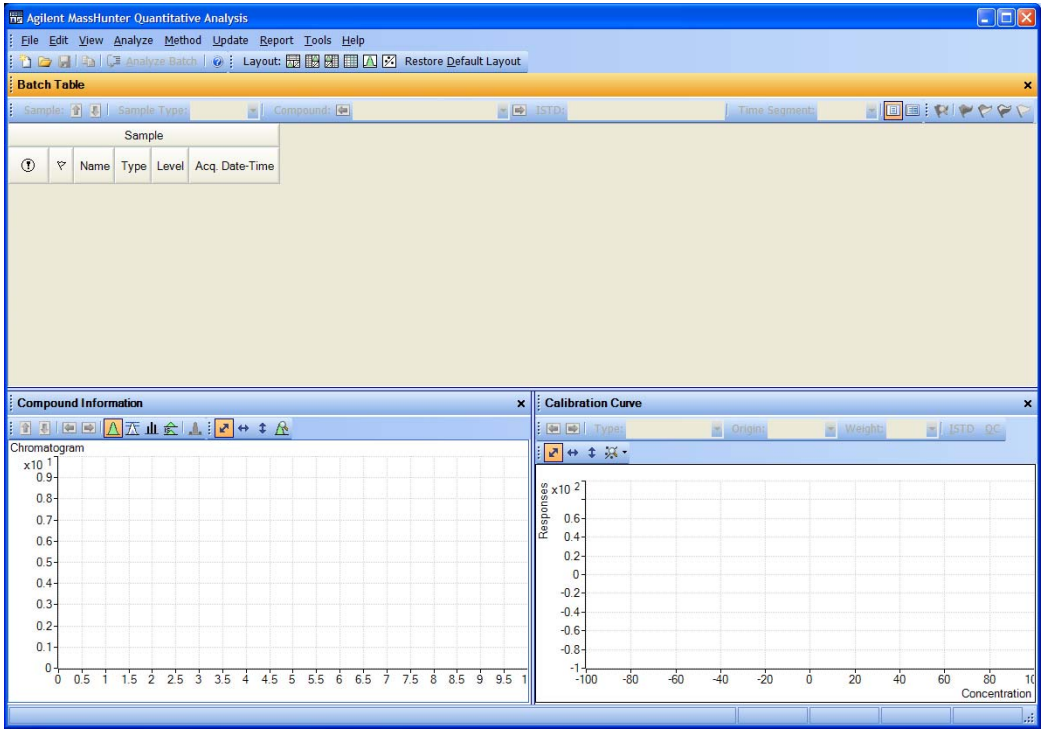
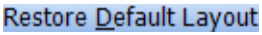
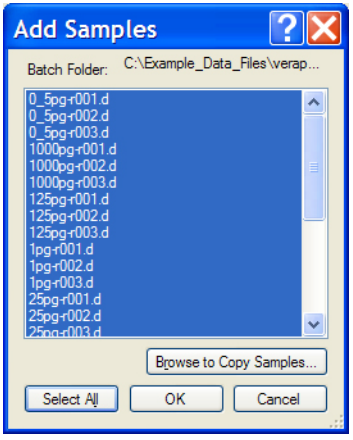


Figure 5 Default layout

Steps	Detailed instructions	Comments
	b Click **File > New Batch**. The system opens the **New Batch** dialog box. **c** Navigate to the folder \ ***Your Directory*** **Verapamil-targetedMSMS**\. **d** Type the batch filename ***iii*_Test_01** and click **Open**.	• If the default layout is not present, click **Restore Default Layout** on the toolbar before creating a new batch. 
2 Add all the samples in the **Verapamil** folder to the batch.	**a** Click **File > Add Samples**: The system displays the **Add Samples** dialog box. **b** Click **Select All** to select all samples, and then click **OK** to add them to the batch. The **Batch Table** is no longer empty. It now contains the calibration and blank samples. See Figure 6. 	• Note that five of the files are blanks and the other files are all calibration files at different calibration levels.

Steps	Detailed instructions	Comments

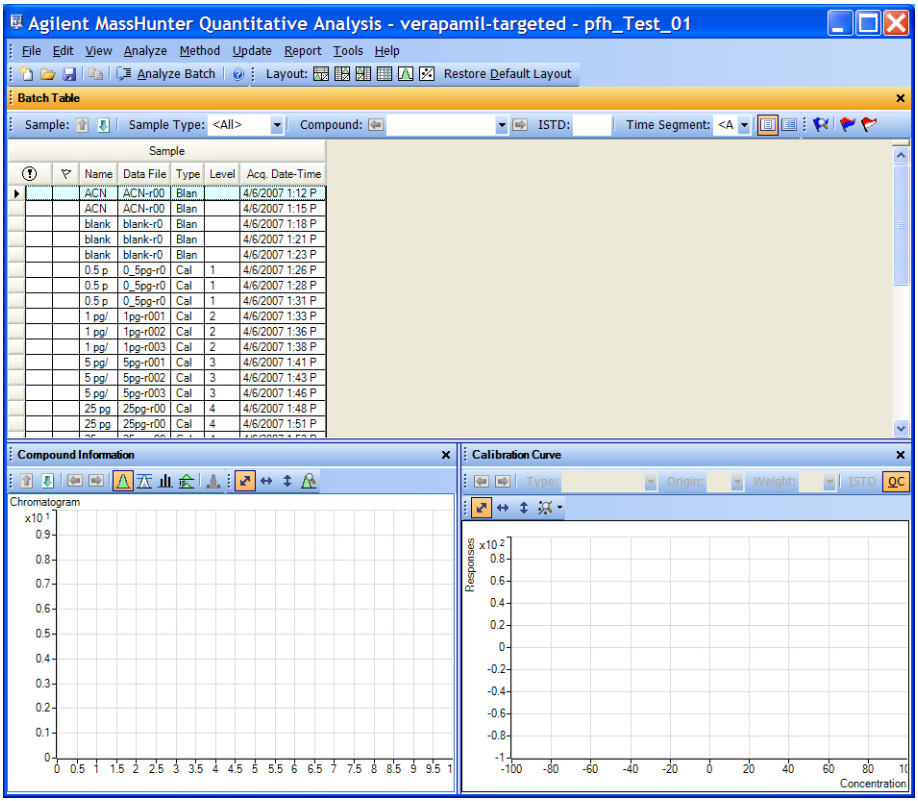
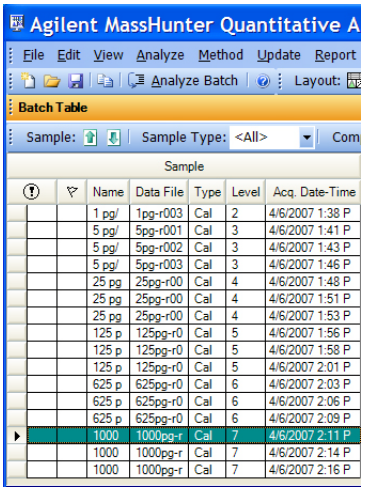
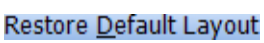


Figure 6 Batch Table containing Verapamil samples before quantitation

Task 2. Set Up a New Method for the Batch

This task shows you how to set up a new quantitation method based on the calibration data file with the highest concentration of sample.

Steps	Detailed instructions	Comments
1 Create a new method from acquired Q-TOF data. • Use the calibration data file with the highest signal.	**a** Use the cursor to highlight the calibration standard that has the highest concentration level, as shown in the figure below.	• Using a sample with strong signals for the compounds, such as a high-concentration calibration sample, lets the program create a method with the appropriate retention times and qualifier ratios.
		
	b Click **Method > Edit** to switch to method editing mode. The **Method Tasks** appear in the column to the left of the View, as shown in Figure 7.	• Note that Figure 7 shows the default layout for method editing. • If the default layout is not present, click **Restore Default Layout** on the toolbar before creating a new method in the next step. 

Steps	Detailed instructions	Comments

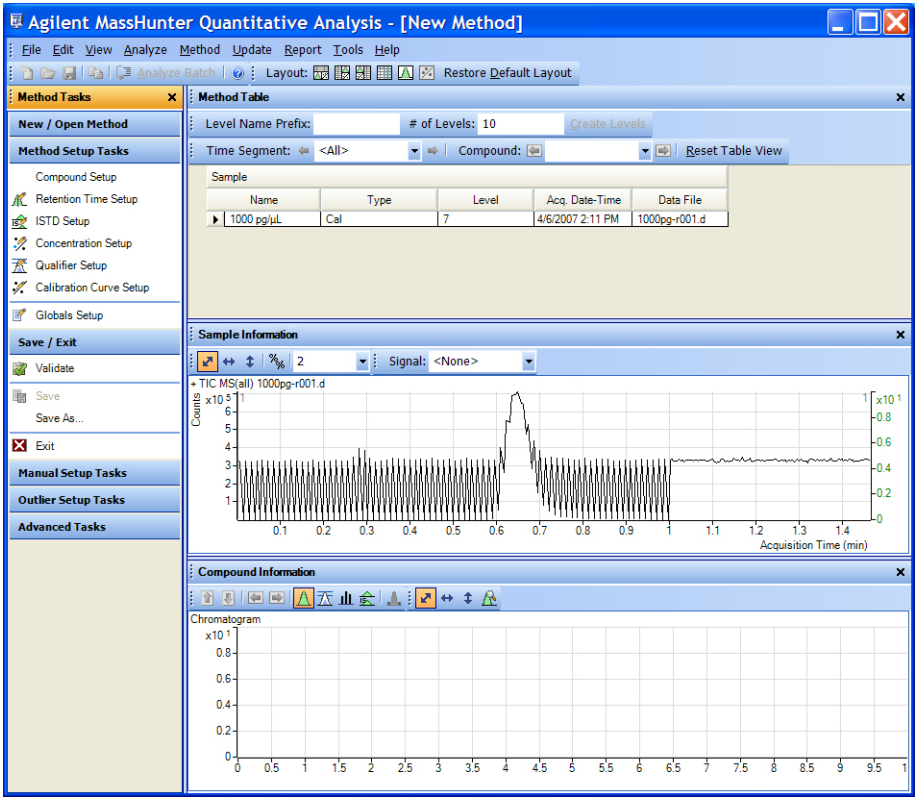
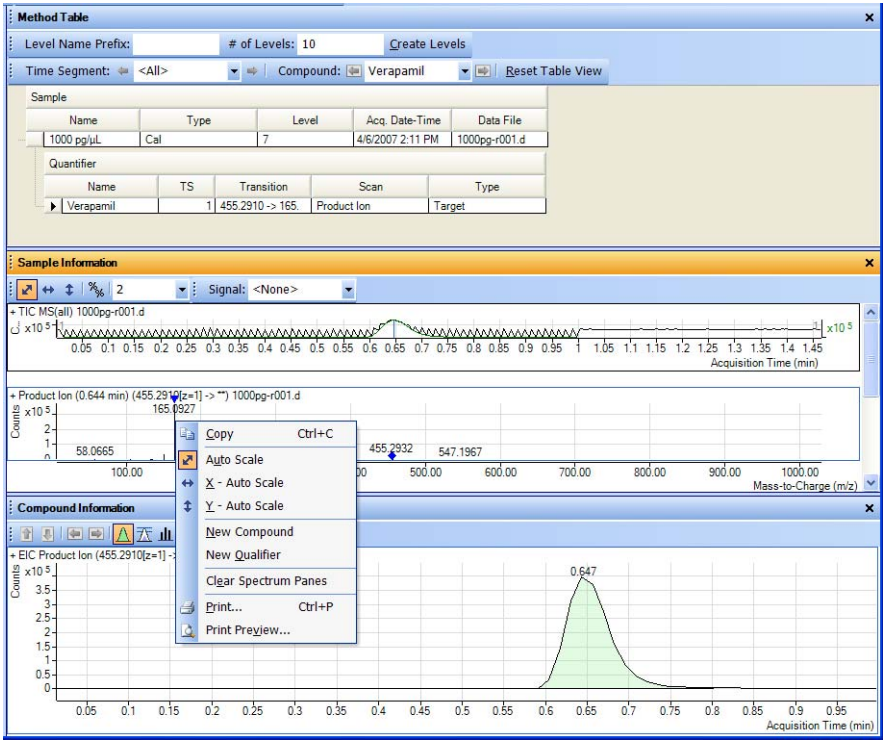


Figure 7 Method Edit mode

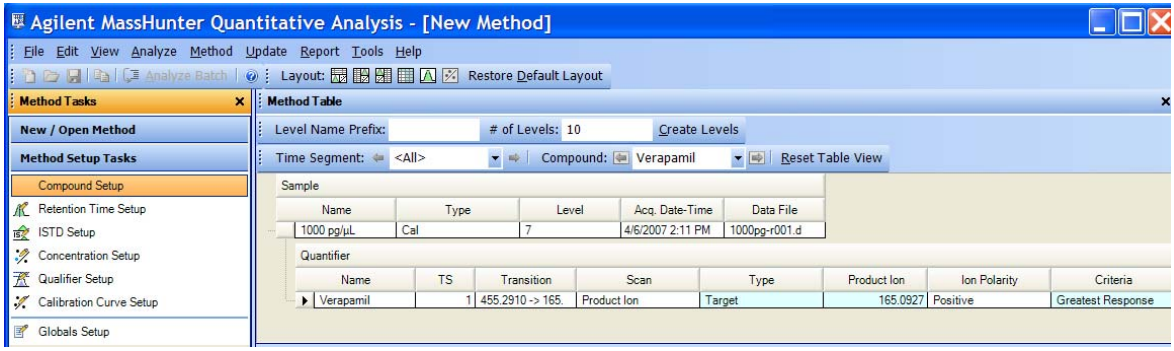
Steps	Detailed instructions	Comments
	c Under **Method Tasks** in the sidebar to the left of the **Method Table**, click **New/Open Method > New Method using Manual Setup**. **d** In the **Sample Information** window, click the middle of the peak. Right-click and click **Extract Spectrum**. • The spectrum "+ Product Ion (0.644 min)(455.2910[z=1] -> **)" is displayed. **e** Click the largest ion, 165.0927. Right-click that location and click **New Compound**. **f** Type `Verapamil` as the **Name** in the **Method Table**.	• The figure below shows the shortcut menu in the **Sample Information** window that is used to add a compound to the method.



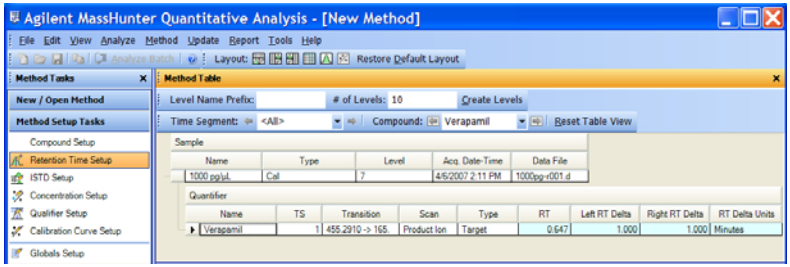
Task 3. Set Up Target Compounds

With this task you learn to inspect the product ions and the RT data for the new quantitation method, which you can change for individual target compounds.

Steps	Detailed instructions	Comments
1 Check the new quantitation method created from the **Sample Information** window for the product ion.	**a** Under **Method Tasks** in the sidebar to the left of the **Method Table**, click **Method Setup Tasks > Compound Setup**.	



Steps	Detailed instructions	Comments
	b To inspect the retention time set from the spectrum, click **Method Setup Tasks > Retention Time Setup**.	• You can modify data fields in blue for individual compounds.

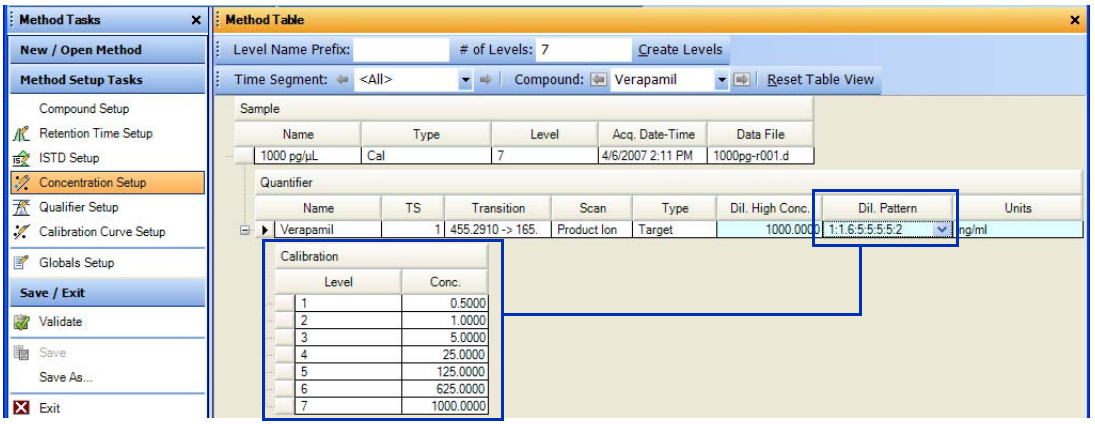


Task 4. Set Up Quantitation

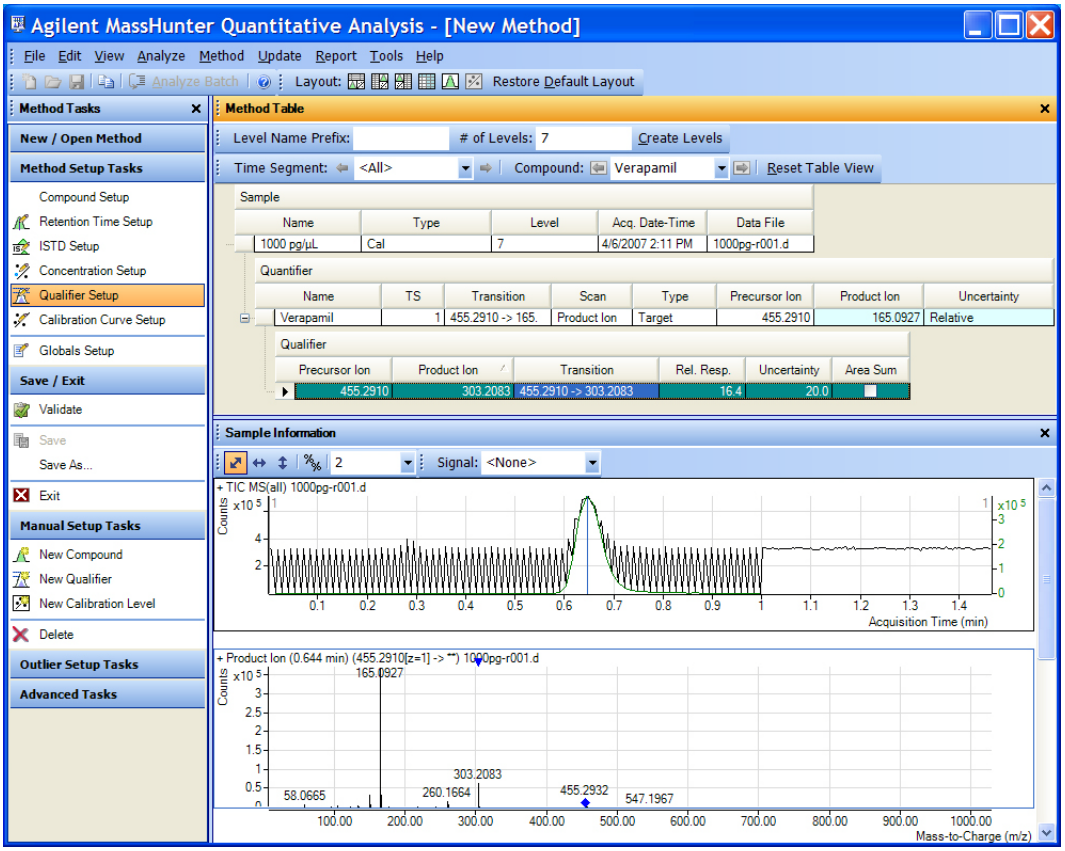
This task presents instructions for setting up the quantitation parameters for the method's:

- Calibration levels
- Qualifier ions
- Calibration curve fit

Steps	Detailed instructions	Comments
1 Create five calibration levels for each compound. • Set the highest concentration for Verapamil of 125. • Set a Dilution Pattern of 1:1.6:5:5:5:5:2 for Verapamil. • Compare the concentrations for the seven levels with the Dilution Pattern.	**a** Click **Method Setup Tasks > Concentration Setup**, and type `1000.000` in the **Dil. High Conc**. column for Verapamil. **b** Type `1:1.6:5:5:5:5:2` in the **Dil. Pattern** column for Verapamil. **c** Make sure **Level Name Prefix** is empty and **# of Levels** is **7** in the **Serial Dilution** toolbar.	
	d Click **Create Levels**. **e** Compare the newly created calibration levels with Dilution High Concentration and Dilution Pattern.	



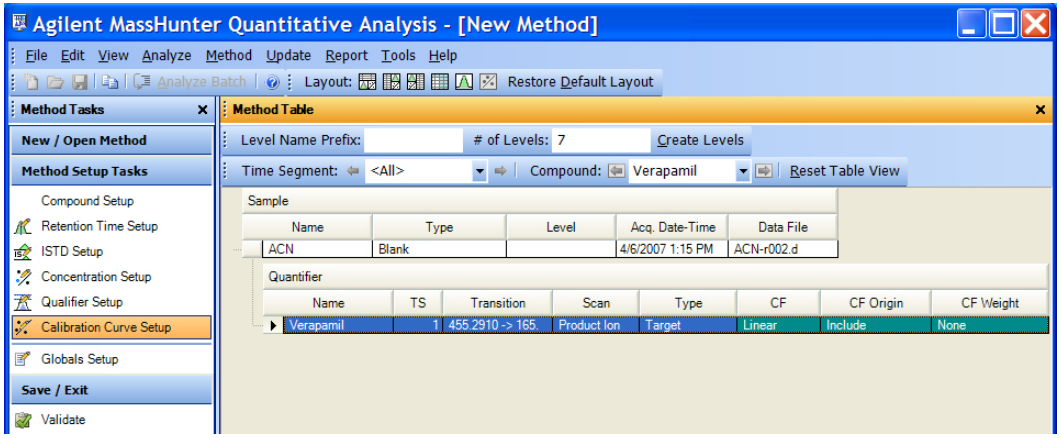
Steps	Detailed instructions	Comments
2 Set up qualifier ions and a calibration curve. • Review the Qualifier setup parameters. • Change the **CF Origin** to **Include**.	**a** Select the spectrum "+ Product Ion (0.644 min)(455.2910[z=1] -> **) 1000 pg-r001.d" in the **Sample Information** window. **b** Click 303.2083. Right-click that location and click **New Qualifier**.	• You can select more than one qualifier ion. • A blue triangle indicates the selected *m/z* in the spectrum.

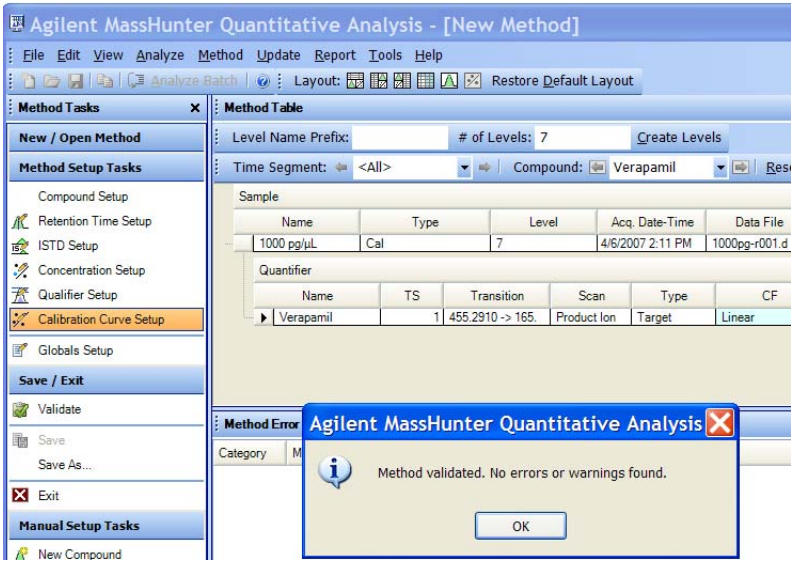


c Click **Method Tasks > Calibration Curve Setup**,

d For the Verapamil compound, change the **CF Origin** to **Include**.

Steps	Detailed instructions	Comments



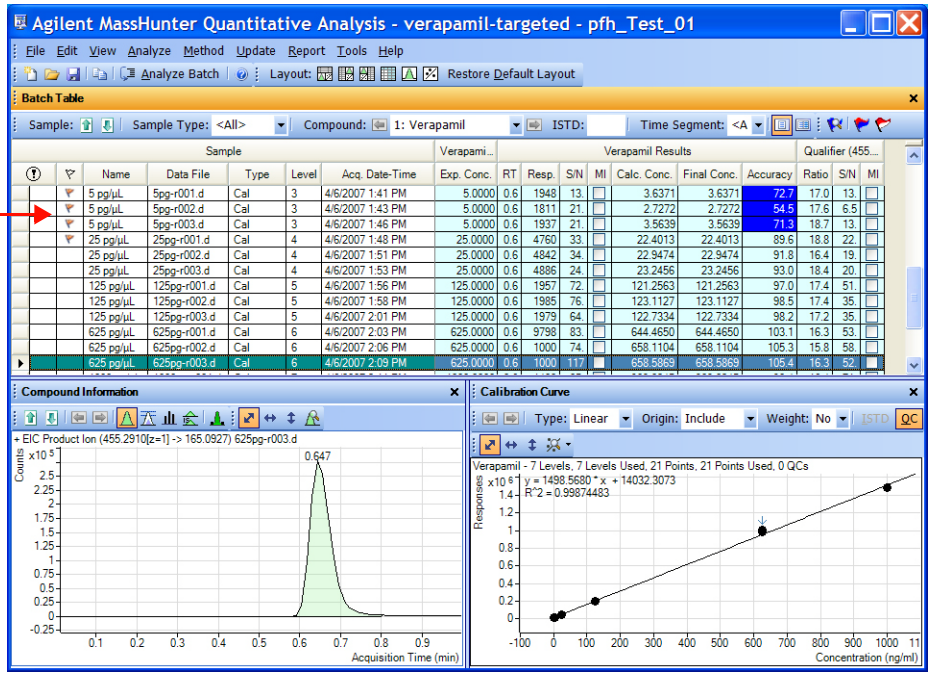
Steps	Detailed instructions	Comments
3 Validate and save the method.	**a** Click **Save/Exit > Validate** to validate the method setup.	• You can view any validation errors that do occur at the bottom of the screen.
		
	b After the validation message appears, click **OK.**	
	c Click **Save/Exit > Exit**, and click **Yes** to the **Would you like to apply this method to the batch?** prompt.	

Task 5. Analyze and Save the Batch

In this exercise you automatically quantitate the batch and then save the results.

Steps	Detailed instructions	Comments
1 Analyze the batch, and inspect the results for each compound. • Examine the Quantitation Message(s), which identify samples with no quantitated signals. • Examine the outlier flag messages.	**a** Click the **Analyze Batch** icon Analyze Batch in the toolbar to start batch analysis. **b** Pass the cursor over the flags for the first 2 calibration standards.	• Note that three calibration standards have outlier flags for **Accuracy**.
2 Save the batch.	**a** Click **File > Save Batch**. **b** Click **File > Close Batch** to close the batch.	

Outlier flag messages



Exercise 3
Review Quantitation Results

The tasks in this exercise show you how to inspect the sample and compound data in a batch file, customize result layouts, export your data to Microsoft Excel, and preview and print the data.

The **DrugsOfAbuse** batch is used in this exercise. The same tasks can be performed with Triple Quad data files, Q-TOF data files, and TOF data files.

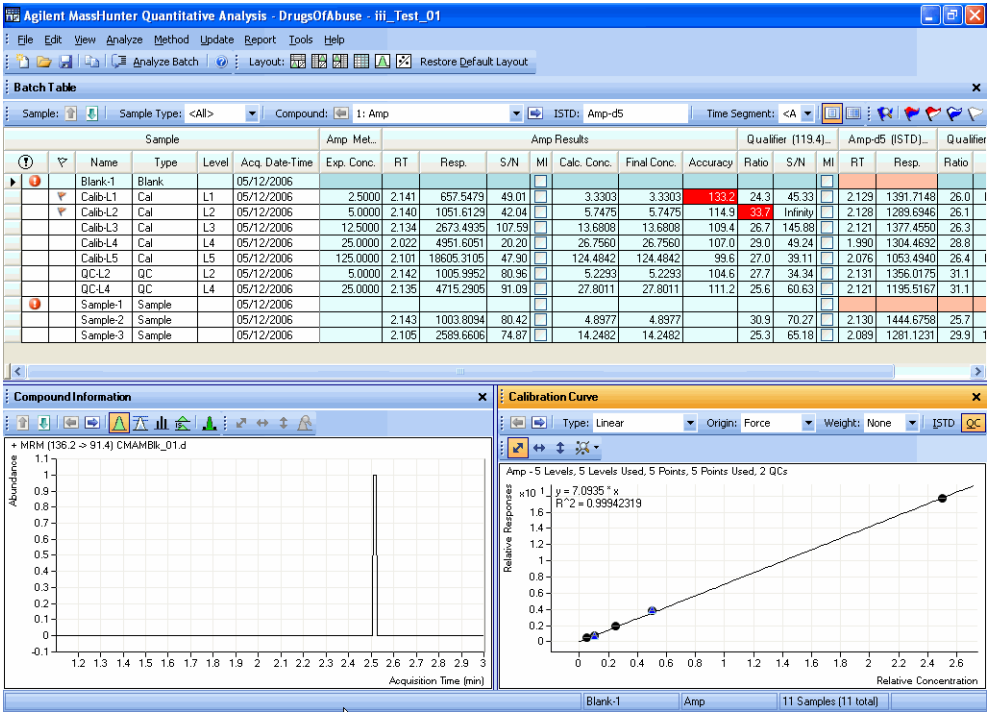
Each exercise is presented in a table with three columns:

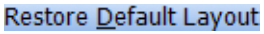
- Steps – Use these general instructions to proceed on your own to explore the program.
- Detailed Instructions – Use these if you need help or prefer to use a step-by-step learning process.
- Comments – Read these to learn tips and additional information about each step in the exercise.

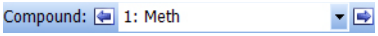
Task 1. Navigate the Batch Table Results

This task shows you how to scroll through your samples and compounds, observing changes in the Batch Table and compound information data. It also shows you how to display various sample types.

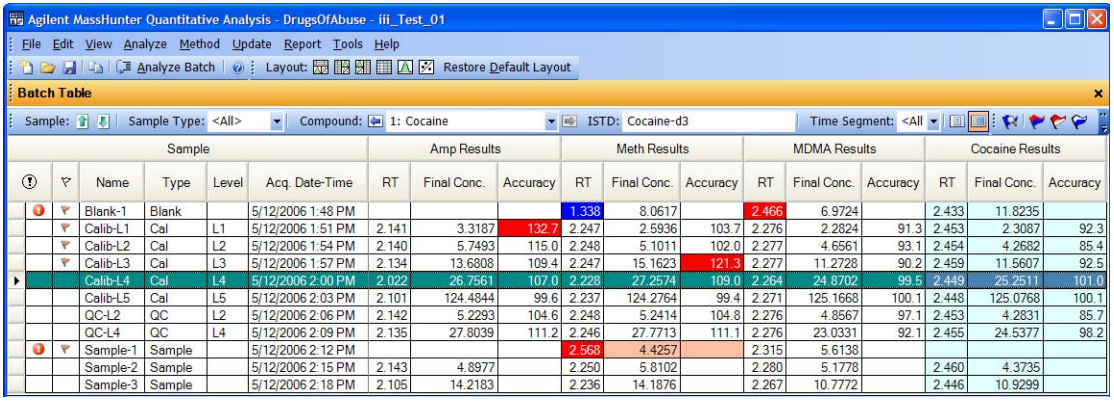
Steps	Detailed instructions	Comments
1 Open the batch file ***iii*_Test_01.batch.xml**., created in Exercise 2.	**a** To start the Quantitative Analysis program, click the **Quantitative Analysis** icon on your Desktop . **b** Click **Open Batch** on the toolbar to display the **Open Batch** dialog box. **c** Navigate to \ ***Your Directory*\ DrugsOfAbuse** and click ***iii*_Test_01.batch.xml.**	• The main view that appears should look like the one below. This is the default layout and contains the default column settings.



Steps	Detailed instructions	Comments
2 (Optional) If you see a different layout than the one in the figure on the previous page... • If fewer than three windows are present in the main view, or they are in a different arrangement, restore the default layout. • If the column settings are different, restore the default column settings. • If panes other than the Chromatogram pane are present in the **Compound Information** window, hide the other panes.	• To restore the default layout, click **Restore Default Layout** on the toolbar before scrolling from sample to sample.  • To restore the default column settings, right-click anywhere in the **Batch Table** window and click **Restore Default Columns**. • To hide extra panes, click the highlighted icons other than the Show/Hide Chromatogram icon  in the Compound Information toolbar.	• The default layout is set at the factory and cannot be changed. If you want to create your own layout, see "Task 2. Change Result Window Layouts" on page 49.
3 Scroll from sample to sample until you reach the end of the **Batch Table**, and then return to Cal-L5. • Use the Next Sample and Previous Sample arrows on the toolbar  . • Note the changes in the **Batch Table** and **Compound Information** of amphetamine for each sample. • Select sample **Calib_ L4** in the **Batch Table** to view the **Batch Table** and **Compound Information** changes.	**a** Click the **Next Sample** arrow  in the Batch Table Standard toolbar until the system displays the desired sample. Inspect the changes in the **Compound Information** window. **b** To return to Cal-L5, click the **Previous Sample** icon  in the Batch Table Standard toolbar. **c** Select any cell in the row for sample **Calib_L4** in the **Batch Table** window to view the changes.	• Note the linkage between the highlighted data file in the **Batch Table** and the chromatogram in the **Compound Information** window.

Steps	Detailed instructions	Comments
4 Scroll from compound to compound through all four compounds. • Use the Next Compound and Previous Compound arrows on the toolbar.  • Review the differences in the **Batch Table**, **Compound Information**, and **Calibration Curve** windows between the compounds. • Select **Cocaine** from the list.	**a** Click the **Next Compound** or **Previous Compound** arrow in the toolbar until the system displays the desired compound. **b** Inspect the changes in the **Batch Table**, **Compound Information** and **Calibration Curve** windows. **c** Click the down arrow next to the **Compound** list. **d** Click **Cocaine**.	

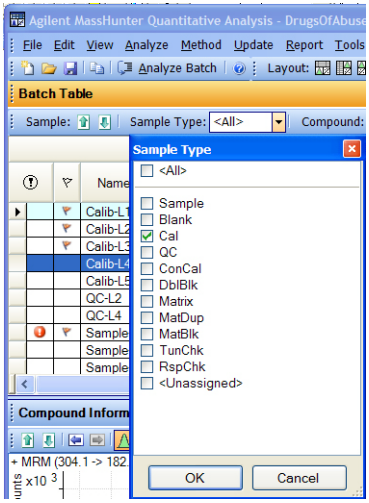
Steps	Detailed instructions	Comments
5 Examine results for multiple compounds. • View the RT for each compound for the Cal-L4 sample. • After reviewing the results for all the compounds, return to viewing the cocaine results.	**a** Click the **Multiple Compound View** icon in the toolbar to display the quantitation results for all target compounds. You can also click **View > Batch Table Layout > Multiple Compound View**. **b** Click the Cal-L4 cell, and note the difference in **RT** in the **Compound Information** window for each compound.	A different set of columns is displayed when you are in Multiple Compound View mode versus Single Compound View mode. If you add a column to the table when you are in Multiple Compound View mode, that change is not automatically made in the Single Compound View mode.



Sample						Amp Results			Meth Results			MDMA Results			Cocaine Results		
		Name	Type	Level	Acq. Date-Time	RT	Final Conc.	Accuracy	RT	Final Conc.	Accuracy	RT	Final Conc.	Accuracy	RT	Final Conc.	Accuracy
		Blank-1	Blank		5/12/2006 1:48 PM				1.338	8.0617		2.466	6.9724		2.433	11.8235	
		Calib-L1	Cal	L1	5/12/2006 1:51 PM	2.141	3.3187	132.7	2.247	2.5936	103.7	2.276	2.2824	91.3	2.453	2.3087	92.3
		Calib-L2	Cal	L2	5/12/2006 1:54 PM	2.140	5.7493	115.0	2.248	5.1011	102.0	2.277	4.6561	93.1	2.454	4.2682	85.4
		Calib-L3	Cal	L3	5/12/2006 1:57 PM	2.134	13.6808	109.4	2.247	15.1623	121.3	2.277	11.2728	90.2	2.459	11.5607	92.5
▸		Calib-L4	Cal	L4	5/12/2006 2:00 PM	2.022	26.7561	107.0	2.228	27.2574	109.0	2.264	24.8702	99.5	2.449	25.2511	101.0
		Calib-L5	Cal	L5	5/12/2006 2:03 PM	2.101	124.4844	99.6	2.237	124.2764	99.4	2.271	125.1668	100.1	2.448	125.0768	100.1
		QC-L2	QC	L2	5/12/2006 2:06 PM	2.142	5.2293	104.6	2.248	5.2414	104.8	2.276	4.8567	97.1	2.453	4.2831	85.7
		QC-L4	QC	L4	5/12/2006 2:09 PM	2.135	27.8039	111.2	2.246	27.7713	111.1	2.276	23.0331	92.1	2.455	24.5377	98.2
		Sample-1	Sample		5/12/2006 2:12 PM				2.568	4.4257		2.315	5.6138				
		Sample-2	Sample		5/12/2006 2:15 PM	2.143	4.8977		2.250	5.8102		2.280	5.1778		2.460	4.3735	
		Sample-3	Sample		5/12/2006 2:18 PM	2.105	14.2183		2.236	14.1876		2.267	10.7772		2.446	10.9299	

c To return to the display of detailed quantitation results for the selected target compound, click the **Single Compound Display** icon in the toolbar.

d If necessary, click the down arrow next to the **Compound** list, and click **Cocaine**.

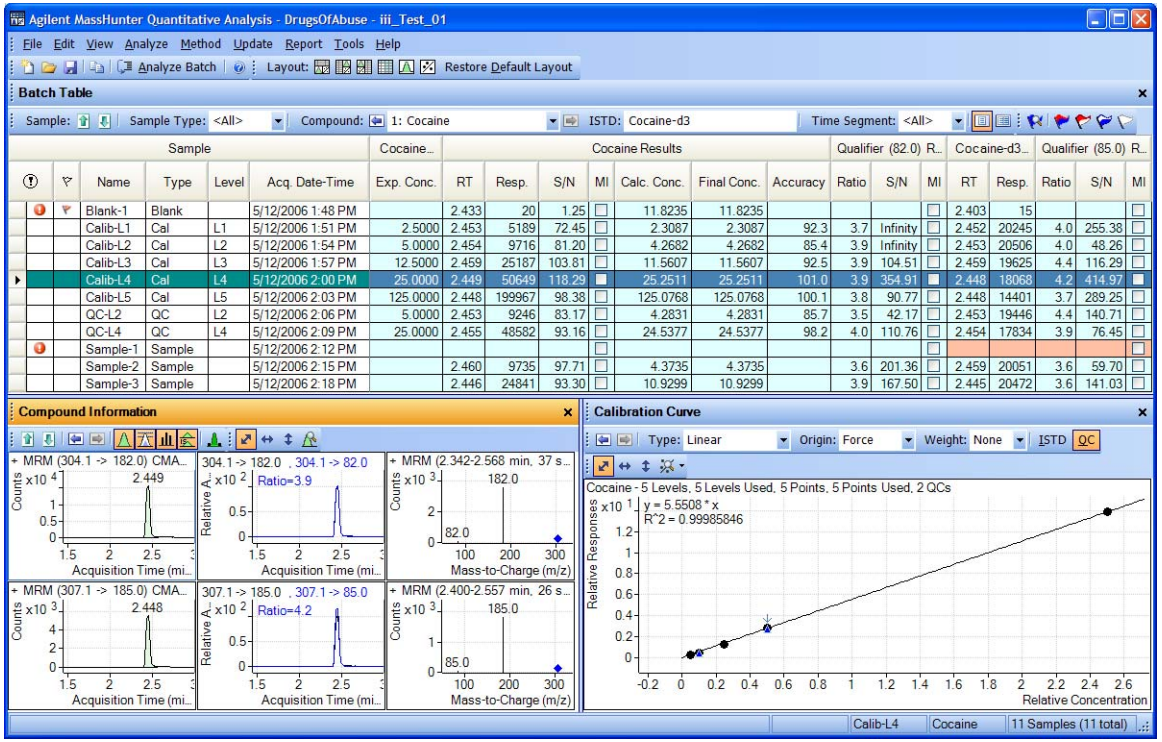
Steps	Detailed instructions	Comments
6 View selected sample types. • Display only the calibration standards. • Then display all sample types.	**a** Click the down arrow in the **Sample Type** dropdown list. The **Sample Type** dialog box is displayed. **b** Clear the **<All>** check box and mark the **Cal** check box.  **c** Click **OK**. The **Batch Table** should contain only the **Cal** standards for cocaine. **d** Click the down arrow in the **Sample Type** dropdown list. **e** Click **<All>**, then click **OK**. The system marks all the check boxes and displays all sample types.	

Task 2. Change Result Window Layouts

This task shows you how to customize your layout using the toolbar icons and how to recreate the default layout.

Steps	Detailed instructions	Comments
1 Use layout icons on the toolbar to position the **Batch Table**, **Compound Information**, and **Calibration Curve** windows. • The default layout is called Table Top because the Batch Table is at the top of the main view. • Change the layout to Table Left, then to Table Right. • Return to the Table Top layout.	**a** Click the **Layout – Table Left** icon in the toolbar  . **b** Click the **Layout – Table Right** icon in the toolbar  . **c** Click the **Layout – Table Top** icon  .	
2 Use layout icons on the toolbar to maximize each individual window: • Table • Compound information • Calibration curve • Return to the default layout	**a** Click the **Maximize Table** icon in the toolbar  . **b** Click the **Maximize Compound Information** icon in the toolbar  . **c** Click the **Maximize Calibration Curve** icon in the toolbar  . **d** To return to the default layout, click the **Restore Default Layout** icon on the toolbar.	
3 Change the panes in the **Compound Information** window for Cal-L4. • Show qualifiers • Show spectra • Show ISTD chromatogram, qualifiers, and spectra	**a** In the **Batch Table**, select the **Cal-L4** row. **b** In the Compound Information toolbar, click the **Show/Hide Qualifiers** icon  . **c** Click the **Show/Hide Spectrum** icon  . **d** Click the **Show/Hide ISTD** icon  . The layout and results look like those in the figure on the next page.	• This step assumes that you started this task with just the Chromatogram pane in the **Compound Information** window. • Changing the layout changes only the position and visibility of the six panes. The panes in the **Compound Information** window are not affected by changing the layout.

Steps	Detailed instructions	Comments



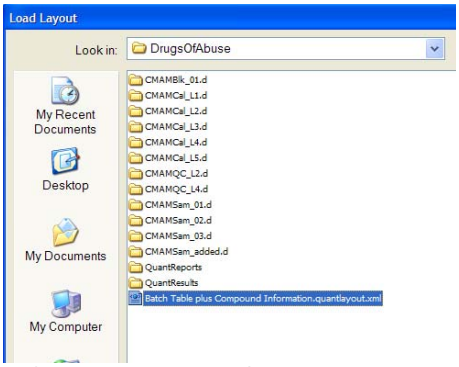
4 Save the default layout without the calibration curve.

- Save the new layout as **Batch Table plus Compound Information** in the **DrugsOfAbuse** folder.

a Close the **Calibration Curve** window.

b Click **View > Window Layout > Save Layout**.
The system displays the **Save Layout File** dialog box.

c Name the layout file **Batch Table plus Compound Information,** and click **Save**.

Steps	Detailed instructions	Comments
5 Load the newly created layout. • Restore the default layout. • Load the layout **Batch Table plus Compound Information**.	**a** Click **Restore Default Layout** on the toolbar. **b** Click **View > Window Layout > Load Layout**. The system displays the **Load Layout** dialog box.  **c** Click **Batch Table plus Compound Information** and click **Open**. The results window should now look like Figure 8.	

Steps	Detailed instructions	Comments

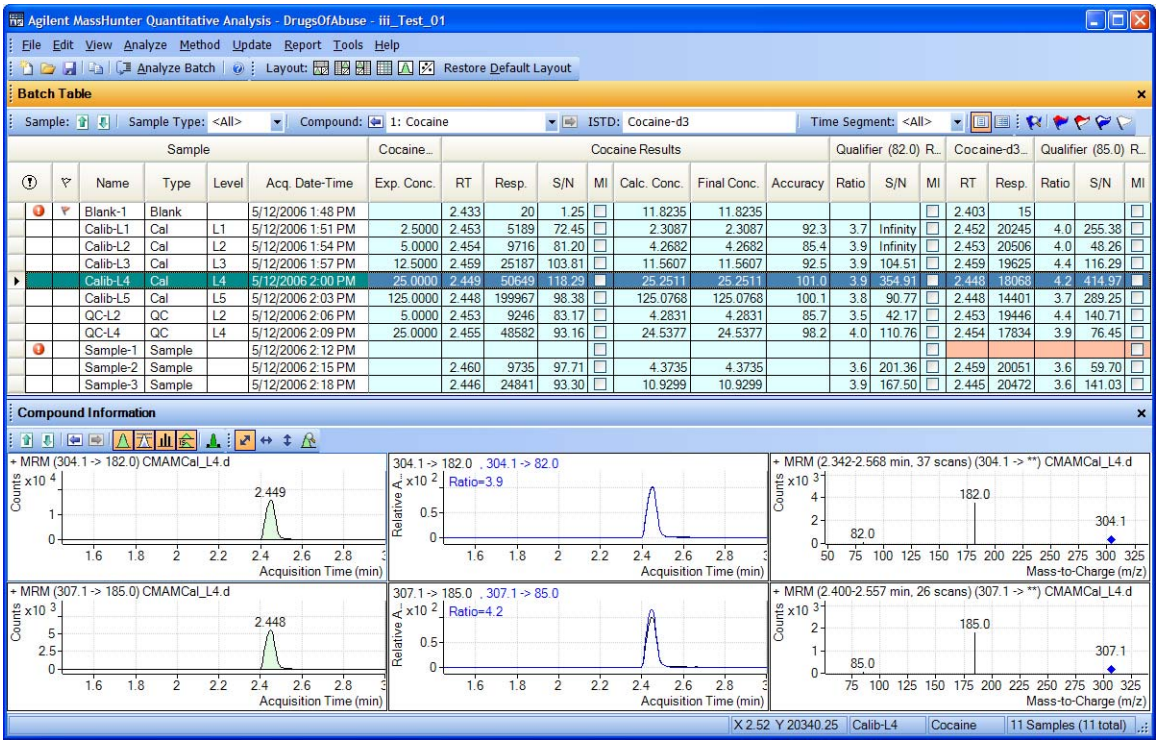
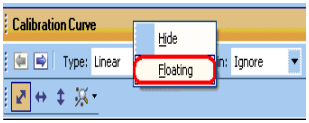


Figure 8 Results window

Steps	Detailed instructions	Comments
6 Create the layout as shown in Figure 9 on page 53, with the **Calibration Curve** and **Compound Information** windows floating. Hint: More than the **Batch Table** is on the left.	**a** Restore the default layout (click **Restore Default Layout** on the toolbar). **b** Right-click inside the title bar of the **Calibration Curve** window, and then mark the **Floating** check box.	



c Right-click the title bar of the **Compound Information** window, and then mark the **Floating** check box.
d Resize the windows to match the layout in Figure 9.

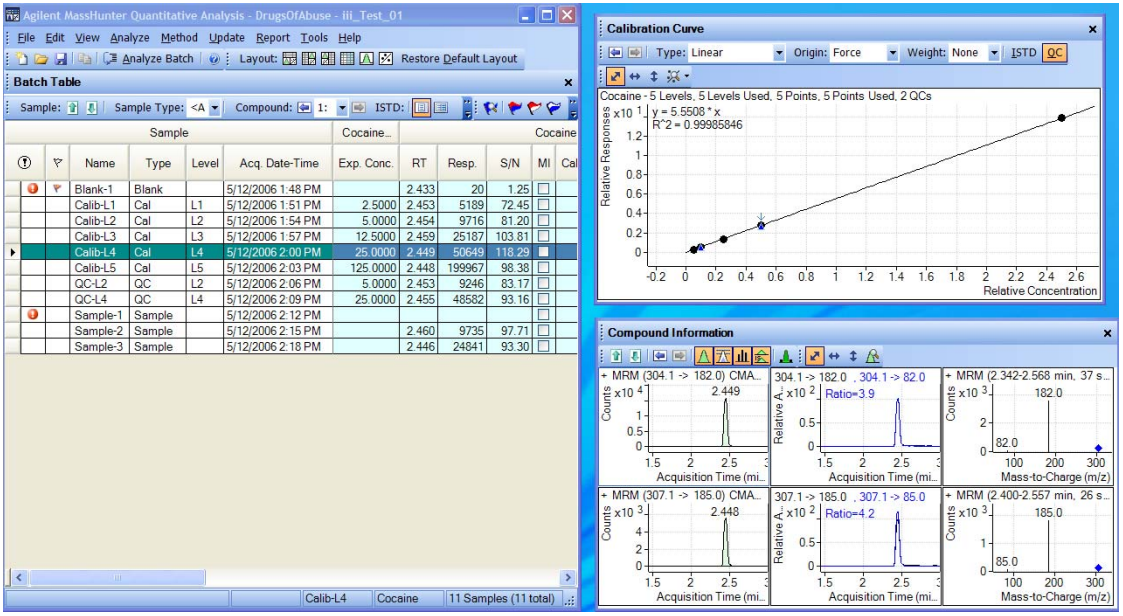


Figure 9 Display with Calibration Curve and Compound Information windows floating

Steps	Detailed instructions	Comments
	e Right-click inside the title bar of the **Compound Information** window, and then clear the **Floating** check box. **f** Resize the windows to match the layout in Figure 10.	

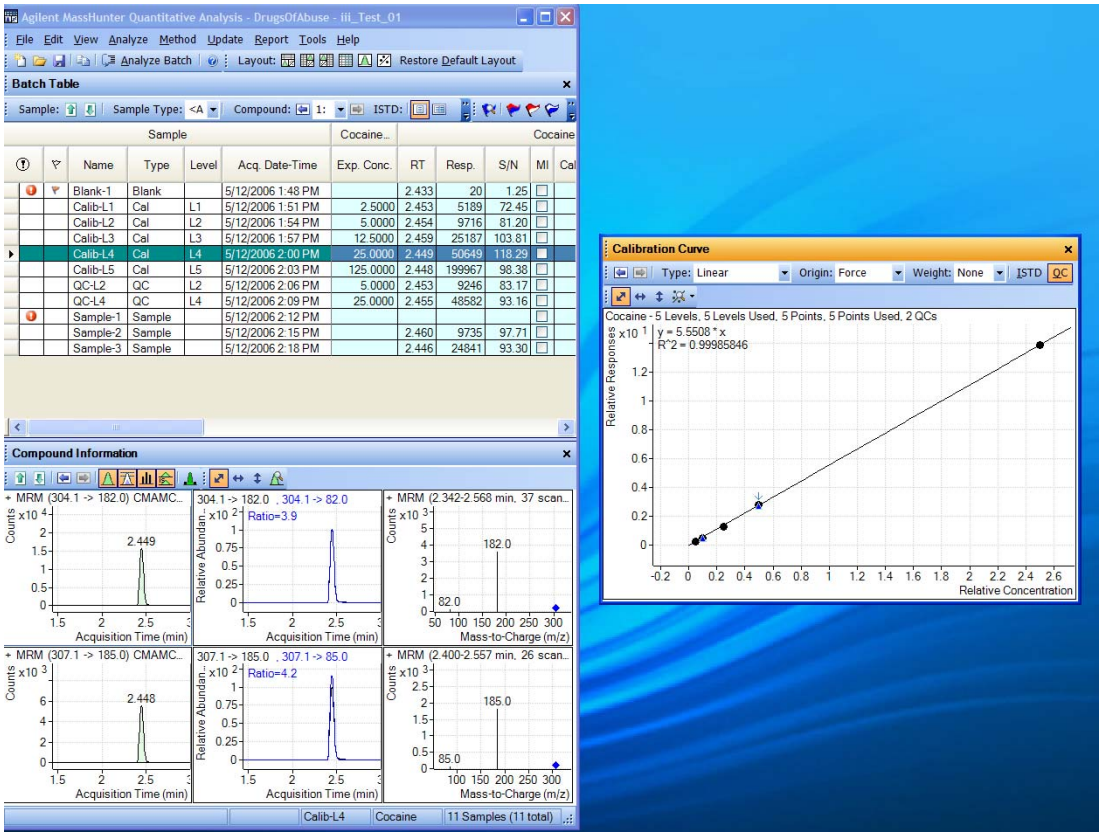


Figure 10 Resized window

Steps	Detailed instructions	Comments
	g Right-click inside the title bar of the **Calibration Curve** window, and clear the **Floating** check box. **h** Move the **Compound Information** window so that the layout corresponds to the one pictured at the start of the task.	
7 Recreate (do not restore) the default layout. • In this step you learn to recreate layouts without using the layout icons or **Restore Default Layout**.	**a** Maximize the program main view.	• You must anchor the **Calibration Curve** window first, and then the **Compound Information** window, to recreate the default layout. • If after anchoring the two windows, the calibration curve is on the left side, you can right-click the title bar of the **Calibration Curve** window and drag it to the right. A gray rectangle is drawn that shows where this window will be placed within the main view. Drag the calibration curve to the bottom right corner of the main view.

Task 3. Export and Print Results

This exercise shows you how to export your data to a Microsoft Excel file and how to preview and print your Batch Table and compound information data.

Steps	Detailed instructions	Comments
1 Export the batch file ***iii*_Test_01**. • Specify My Documents as the destination directory. • Use ***iii*_Test_01.xls** as the export file name, where "*iii*" are your initials.	**a** To make the **Batch Table** window active, click the title bar of the **Batch Table** window. **b** Click **File > Export > Export Table**. **c** Select **My Documents** as the destination directory. **d** Type `iii_Test_01.xls` as the export file name. **e** Click **Save**.	

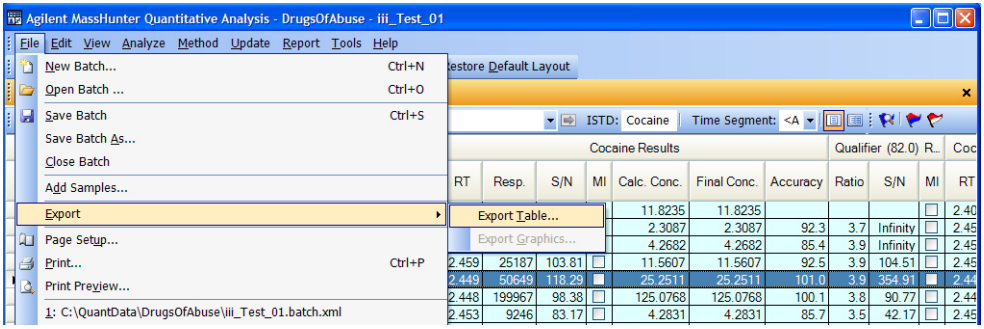
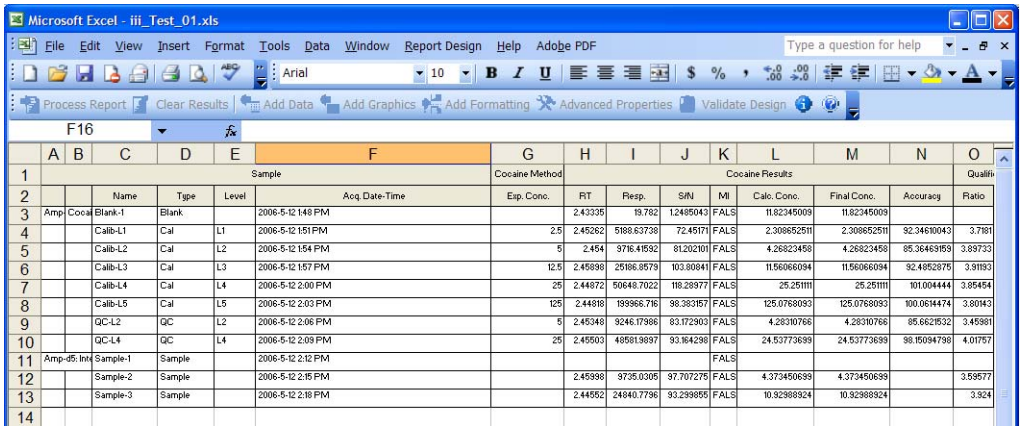


Figure 11 Export results

2 View the batch results as they appear in Excel; then exit Excel. • Note what is exported and what is not.	**a** Start Microsoft Excel. **b** Open **My Documents*iii*_Test_01.xls.** **c** Note what is exported and what is not. **d** Close Excel when you are finished.	

Steps	Detailed instructions	Comments



	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O
1	Sample						Cocaine Method	Cocaine Results							Qualifi
2			Name	Type	Level	Acq. Date-Time	Exp. Conc.	RT	Resp.	S/N	ML	Calc. Conc.	Final Conc.	Accuracy	Ratio
3	Amp	Coca	Blank-1	Blank		2006-5-12 1:48 PM		2.43335	19.782	1.2485043	FALS	11.82345009	11.82345009		
4			Calib-L1	Cal	L1	2006-5-12 1:51 PM	2.5	2.45262	5188.63738	72.45171	FALS	2.30865251	2.30865251	92.34610043	3.7181
5			Calib-L2	Cal	L2	2006-5-12 1:54 PM	5	2.454	9716.41592	81.202101	FALS	4.26823458	4.26823458	85.36469159	3.89733
6			Calib-L3	Cal	L3	2006-5-12 1:57 PM	12.5	2.45898	25186.8579	103.80841	FALS	11.56066094	11.56066094	92.4852875	3.91193
7			Calib-L4	Cal	L4	2006-5-12 2:00 PM	25	2.44872	50648.7022	118.28977	FALS	25.251111	25.251111	101.004444	3.85454
8			Calib-L5	Cal	L5	2006-5-12 2:03 PM	125	2.44818	199366.716	98.383157	FALS	125.0768093	125.0768093	100.0614474	3.80143
9			QC-L2	QC	L2	2006-5-12 2:06 PM	5	2.45348	9246.17986	83.172903	FALS	4.28310766	4.28310766	85.6621532	3.45981
10			QC-L4	QC	L4	2006-5-12 2:09 PM	25	2.45503	48581.9897	93.164298	FALS	24.53773699	24.53773699	98.15094798	4.01757
11	Amp-d5: Int		Sample-1	Sample		2006-5-12 2:12 PM					FALS				
12			Sample-2	Sample		2006-5-12 2:15 PM		2.45998	9735.0305	97.707275	FALS	4.373450699	4.373450699		3.59577
13			Sample-3	Sample		2006-5-12 2:18 PM		2.44552	24840.7796	93.299855	FALS	10.92988924	10.92988924		3.924
14															

Figure 12 Batch table in Excel

3 Preview printouts for Batch Table and Compound Information data.

- Print the Batch Table and Compound Information.
- Save and exit the batch if you are not going to perform Exercise 4 right away.

a Click inside the title bar of the **Batch Table** window, and click **File > Print Preview**.

b Inspect the display of the **Batch Table** in the **Print Preview** window to make sure it looks the way you want it.

c Close the **Print Preview** window.

d When the **Batch Table** is satisfactory, click **File > Print**.

e Repeat steps a-d for the compound information.

f If you are not moving on to Exercise 4, click **File > Save Batch**.

g Click **File > Exit**.

You can also print the **Batch Table** from the **Print Preview** program by clicking the **File > Print** menu item in the **Print Preview** program.

Exercise 4
Use Three Tools to Evaluate Results

In this exercise you will use three tools to help you evaluate and obtain more accurate quantitation results:

- Curvefit Assistant, which calculates all combinations of curves and presents results with an equation and confidence band
- Parameterless integrator, so you don't have to figure out the parameters to change to improve the integration
- Outlier messages to help you easily detect result values that are out of the specified range

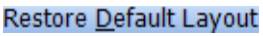
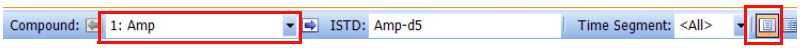
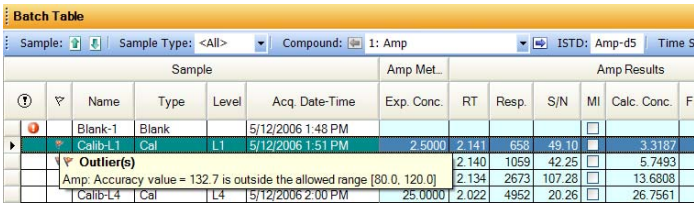
The DrugsOfAbuse batch is used in this exercise. The same tasks can be performed with Triple Quad data files, Q-TOF data files, and TOF data files.

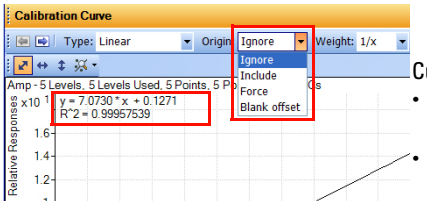
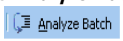
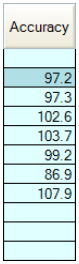
Each exercise is presented in a table with three columns:

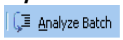
- Steps – Use these general instructions to proceed on your own to explore the program.
- Detailed Instructions – Use these if you need help or prefer to use a step-by-step learning process.
- Comments – Read these to learn tips and additional information about each step in the exercise.

Task 1. Adjust the Calibration Curve Fit

This task shows you how to find the accuracy outlier for a compound, adjust its curve fit, and reanalyze the batch.

Steps	Detailed instructions	Comments
1 If necessary, open the batch file ***iii*_Test_01.batch.xml**. If the batch is already open, skip to step 2.	**a** To start the Quantitative Analysis program, click the **Quantitative Analysis (QQQ)** icon  on your desktop. **b** Click **Open Batch**  on the toolbar to display the **Open Batch** dialog box. **c** Navigate to ***Your Directory*****DrugsOfAbuse** and click ***iii*_Test_01.batch.xml.**	• You can also access the program by clicking **Programs > Agilent > MassHunter Workstation > Quantitative Analysis (QQQ)** from the Start menu. • If the default layout is not present, click **Restore Default Layout** on the toolbar before opening the batch. 
2 Find the accuracy outlier for amphetamine, and change the curve fit. • Set **Origin** to **Ignore**, and **Weight** to **1/y**.	**a** Make sure the **Batch Table** is set to single compound display mode, and the displayed target compound is **Amp**. See boxed portions of the illustration below. 	
	b Point to the cell in the **Calib-L1** row and the **Accuracy** column to display the Outlier message as shown below. 	• Cells containing outliers can be in red (high) or blue (low).

Steps	Detailed instructions	Comments
	c In the **Calibration Curve** window, set **Origin** to **Ignore**, and **Weight** to **1/y.** The program displays a new curve fit formula and R^2 value. 	Curve Fit Origin • **Force** – Forces the curve fit line to go through the origin point (X=0, Y=0). • **Ignore** – Does not force the curve fit line to use the origin point (X=0, Y=0). Curve Fit Weight • **None** – Gives equal weight to all data points. • **1/Y** – Applies the formula 1/Y to the data points. This formula reduces the influence of high Y values while boosting the influence of low Y values.
3 Analyze the batch and inspect the results in the **Batch Table**.	**a** Click the **Analyze Batch** icon in the toolbar  to analyze the batch. **b** Inspect the results in the **Batch Table** after batch analysis. 	
4 Find accuracy outliers, if any, for other compounds.	**a** Click **Next Compound** in the Batch Table toolbar to view individual compounds, such as Cocaine, MDMA, and Met. **b** Examine the quantitation results, especially the values in the **Accuracy** column.	• Note that the Accuracy value for the Calib-L3 standard for methamphetamine is out of the specified range.

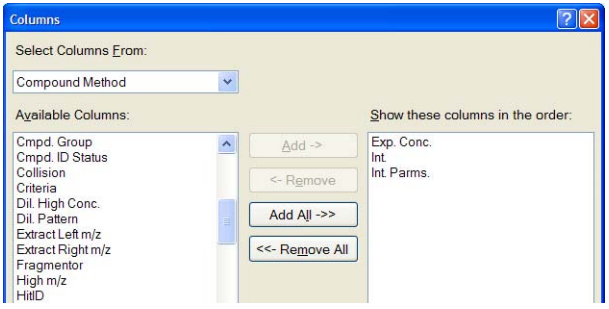
Steps	Detailed instructions	Comments
5 Change the curve fit for methamphetamine, and analyze the batch.	**a** In the **Calibration Curve Fit** window, set **Origin** to **Ignore**, and **Weight** to **1/y**. The Quantitative Analysis program displays a revised curve fit formula and R2 value. **b** Click **Analyze Batch** in the main toolbar  to analyze the batch. The **Batch Table** displays the new results after batch analysis.	

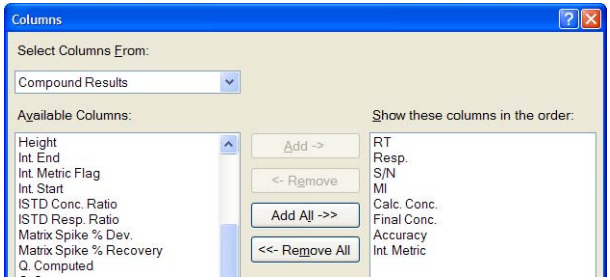
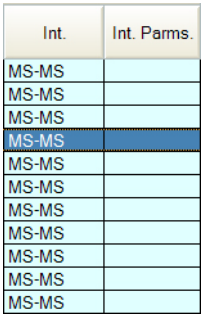
Task 2. Integrate Without Parameters

This task shows you how to inspect data for proper integration. You learn how to perform the following tasks:

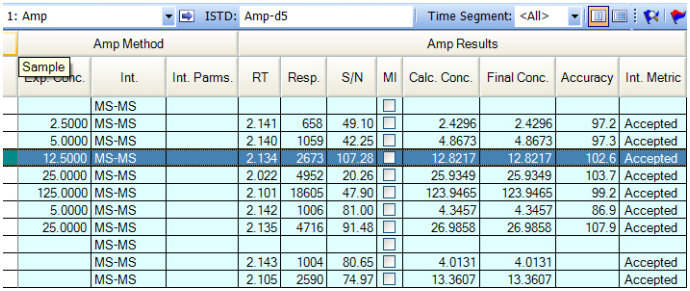
- Add integration columns to the Batch Table
- View default integration values
- Closely examine the chromatogram, looking for such details as:
  - Outlier messages
  - Baseline parameters
  - Peak labels

Steps	Detailed instructions	Comments
1 Add integration columns to the Batch Table. • Add the Integrator Type and Integrator Parameters columns from the Compound Method list. • Add the Integrator Metric column to the Batch Table from the Compound Results list.	**a** Right-click anywhere in the **Batch Table**, and click **Add/Remove Columns**. The system displays the **Columns** dialog box. **b** Select **Compound Method** from the **Select Columns From** drop-down list. **c** Select **Int.** (Integrator Type) and **Int. Parms.** (Integrator Parameters) from the **Available Columns** list, and click **Add**. The Quantitative Analysis program moves the selected columns to the **Show these columns in the order** list.	• This task assumes that the batch, ***iii*_Test_01**, is already open. If it is not, see step 1 in Task 1.



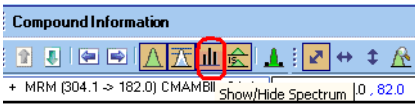
Steps	Detailed instructions	Comments
	d Select **Compound Results** from the **Select Columns From** drop-down list. **e** Select **Int. Metric** (Integrator Metric) from the **Available Columns** list, and click **Add**. The system moves the selected column to the **Show these columns in the order** list. **f** Click **OK**. 	
2 View the default integration values for amphetamine. • View the **Int.** type and **Int. Parms.** columns • View the **Int. Metric** column.	**a** Click **Previous Compound** in the Batch Table toolbar to view amphetamine (**Amp**). **b** Examine the default values in the **Int.** and **Int. Parms** columns in the **Batch Table.** 	• Note that the default integrator used is the MS-MS integrator, which does not need you to enter parameters. That is why the **Int. Parms** column is blank.

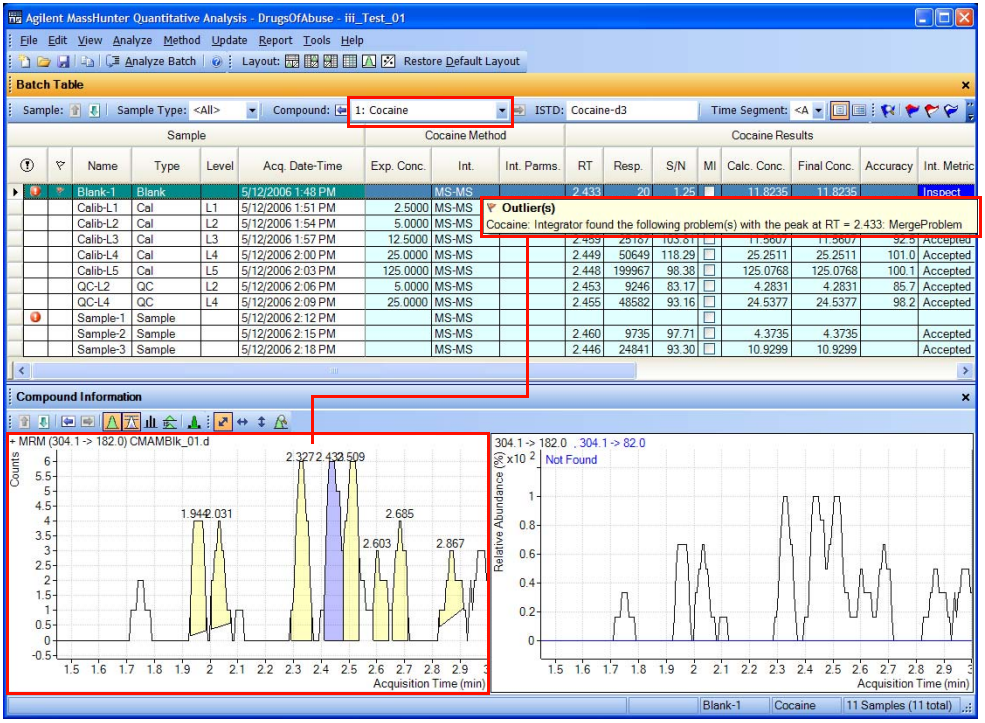
Steps	Detailed instructions	Comments
	c Examine the default values in the **Int. Metric** column in the **Batch Table**.	• These values reflect the default integration quality metric used for the target compound Amp.



1: Amp ISTD: Amp-d5 Time Segment: <All>

Amp Method			Amp Results							
Sample Exp. Conc.	Int.	Int. Parms.	RT	Resp.	S/N	MI	Calc. Conc.	Final Conc.	Accuracy	Int. Metric
	MS-MS					☐				
2.5000	MS-MS		2.141	658	49.10	☐	2.4296	2.4296	97.2	Accepted
5.0000	MS-MS		2.140	1059	42.25	☐	4.8673	4.8673	97.3	Accepted
12.5000	MS-MS		2.134	2673	107.28	☐	12.8217	12.8217	102.6	Accepted
25.0000	MS-MS		2.022	4952	20.26	☐	25.9349	25.9349	103.7	Accepted
125.0000	MS-MS		2.101	18605	47.90	☐	123.9465	123.9465	99.2	Accepted
5.0000	MS-MS		2.142	1006	81.00	☐	4.3457	4.3457	86.9	Accepted
25.0000	MS-MS		2.135	4716	91.48	☐	26.9858	26.9858	107.9	Accepted
	MS-MS					☐				
	MS-MS		2.143	1004	80.65	☐	4.0131	4.0131		Accepted
	MS-MS		2.105	2590	74.97	☐	13.3607	13.3607		Accepted

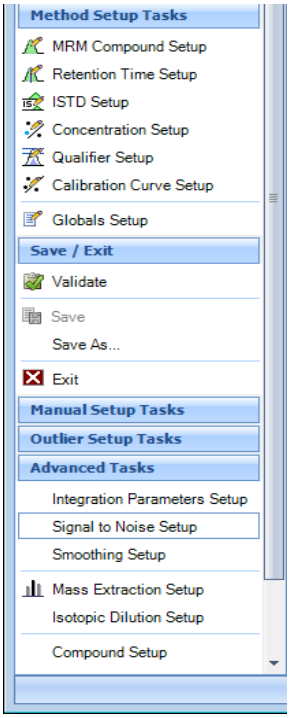
Steps	Detailed instructions	Comments
3 View integration problems for cocaine and MDMA. • Enlarge the chromatogram portion of Compound Information toolbar so that only the quantifier and qualifier chromatograms appear. • Look for outlier messages at the intersection of the **Int. Metric** column and the **Blank-1** sample.	**a** Close the **Calibration Curve** window. **b** To enlarge the chromatogram portion on the Compound Information toolbar, click the **Show/Hide Spectrum** icon.  **c** Also click the **Show/Hide ISTD** icon. **d** Click the **Next Compound** icon in the Batch Table toolbar until the system displays the compound **Cocaine**. **e** Select the **Blank-1** row, and point to the **Int. Metric** column for that row. The system displays any outlier message for that data, as well as the integrated chromatogram for cocaine.	

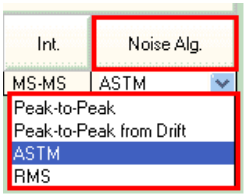
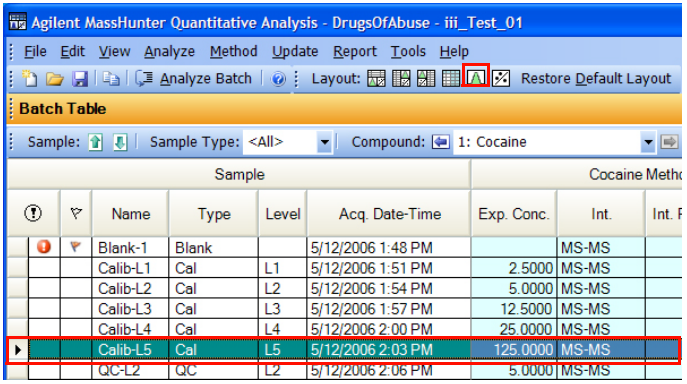


Steps	Detailed instructions	Comments
	f Click the **Next Compound** icon in the Batch Table Standard toolbar or the Previous Compound icon in the Batch Table Standard toolbar until the system displays the compound MDMA. **g** Select the **Blank-1** row, and point to the **Int. Metric** column. The system displays any outlier message for that data, as well as the integrated chromatogram for MDMA.	• The outlier message reads "MDMA: Integrator found the following problems with the peak at RT = 2.4664: Interference Problem." • Note that these colors appear for the integration metric: Green - Accepted Blue - Inspect Red - Rejected • These colors are also reflected in the peak colors.
4 Change the noise algorithm. • Add the Noise Algorithm column from the Compound Method list. • View the values in the **Noise Alg.** and **S/N** columns for amphetamine.	**a** Right-click anywhere in the **Batch Table,** and click **Add/Remove Columns**. The system displays the **Columns** dialog box. **b** Select **Compound Method** from the **Select Columns From** drop-down list. **c** Select **Noise Alg**. (Noise Algorithm Type) from the **Available Columns** list, and click **Add**. The system moves the selected column to the **Show these columns in the order** list. **d** Click **OK**. **e** Click the **Previous Compound** icon in the Batch Table tool bar until the system displays the compound Amp. **f** Examine the values in the **Noise Alg.** and **S/N** (signal-to-noise ratio) columns.	

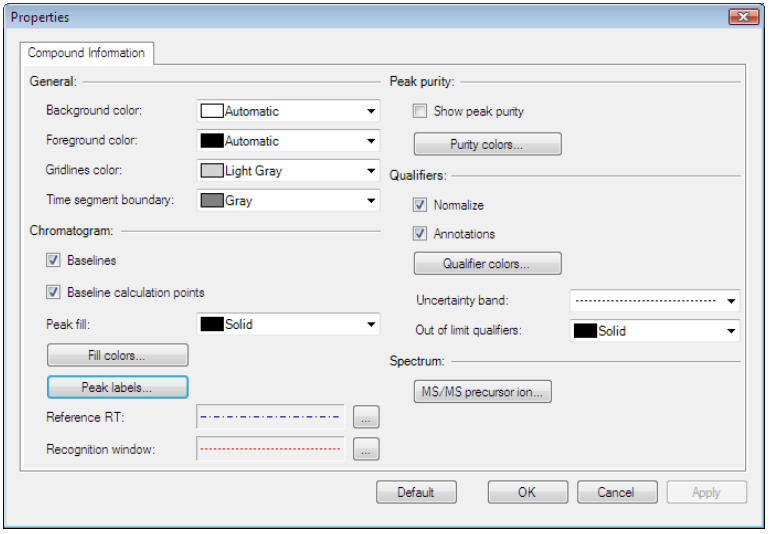
: Amp ISTD: Amp-d5 Time Segment: <A

	Amp Results								Qualifier (119.4)...			Amp
Noise Alg.	RT	Resp.	S/N	MI	Calc. Conc.	Final Conc.	Accuracy	Int. Metric	Ratio	S/N	MI	RT
RMS												
RMS	2.141	658	49.10		2.4296	2.4296	97.2	Accepted	24.3	45.47		2.12
RMS	2.140	1059	42.25		4.8673	4.8673	97.3	Accepted	33.5	Infinity		2.12
RMS	2.134	2673	107.28		12.8217	12.8217	102.6	Accepted	26.7	146.48		2.12
RMS	2.022	4952	20.26		25.9349	25.9349	103.7	Accepted	29.1	49.40		1.99
RMS	2.101	18605	47.90		123.9465	123.9465	99.2	Accepted	27.0	39.22		2.07
RMS	2.142	1006	81.00		4.3457	4.3457	86.9	Accepted	27.7	34.47		2.13
RMS	2.135	4716	91.48		26.9858	26.9858	107.9	Accepted	25.6	60.79		2.12
RMS												
RMS	2.143	1004	80.65		4.0131	4.0131		Accepted	30.9	70.54		2.13
RMS	2.105	2590	74.97		13.3607	13.3607		Accepted	25.3	65.40		2.08

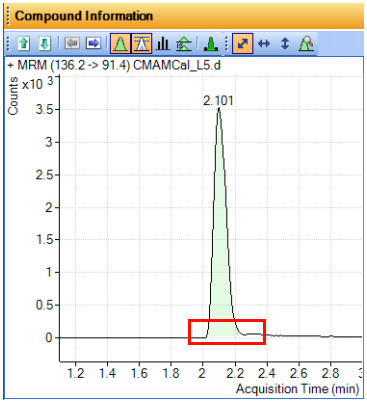
Steps	Detailed instructions	Comments
5 Practice changing the noise algorithm from RSM to ASTM for amphetamine in the method. • Exit, but don't save, the method.	**a** Click **Method > Edit** to switch to method editing mode. **b** Click **Method Tasks > Advanced Tasks > Signal to Noise Setup**. The system displays the integrator parameters in the **Method Table**.  **c** Click the **Noise Alg.** column for Amp in the **Method Table**. A list of available noise algorithms appears. **d** Click **ASTM**.	

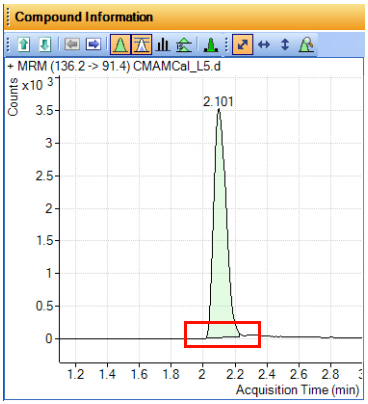
Steps	Detailed instructions	Comments
	 **e** Click **Method Tasks > Save/Exit > Exit**. **f** Click **No** to the exit prompt **Would you like to apply this method to the batch?** The system displays Batch Analysis mode.	
6 Turn the baseline (highest concentration standard) off and then back on for amphetamine. • Make sure that only the Compound Information pane is visible in the window. • Compare the two chromatograms: one with the baseline on and the other with it off.	**a** Select sample **Calib-L5** (if it is not already selected), and click the **Maximize Compound Information** icon in the toolbar. 	• Notice that the baseline is drawn in for the quantifier chromatogram as the default setting.

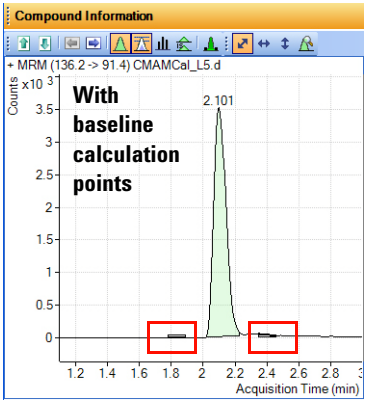
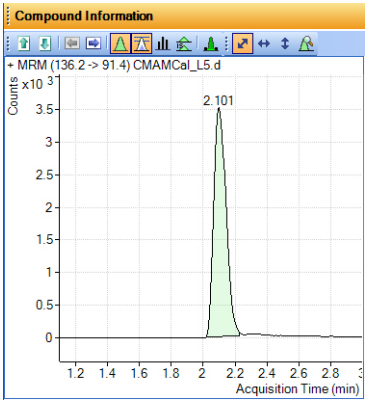
Steps	Detailed instructions	Comments
	b Right-click either of the chromatograms to open the shortcut menu. Click **Properties** at the bottom of the shortcut menu to open the **Properties** dialog box.	



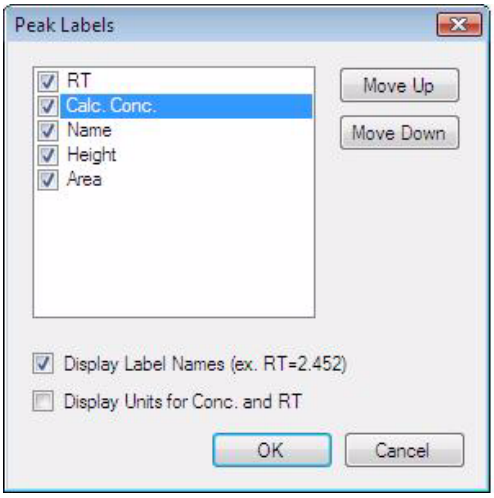
	c Clear the **Baselines** check box in the **Properties** dialog box. Click the **Apply** button and observe the peak without the baseline.	• Notice that the baseline disappears after clearning the baseline check box.

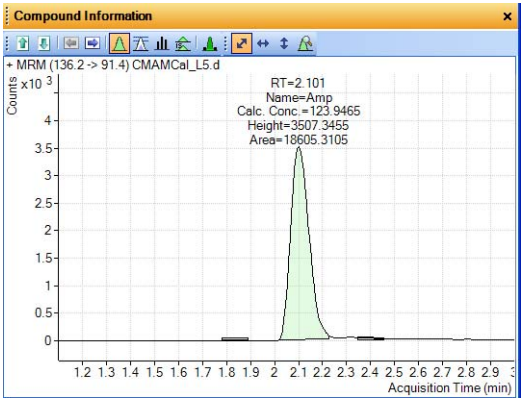


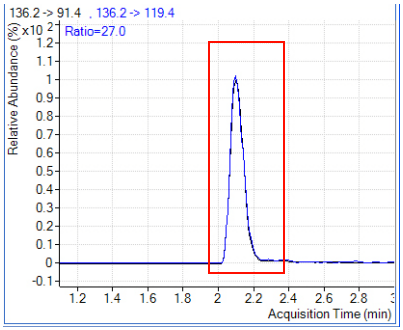
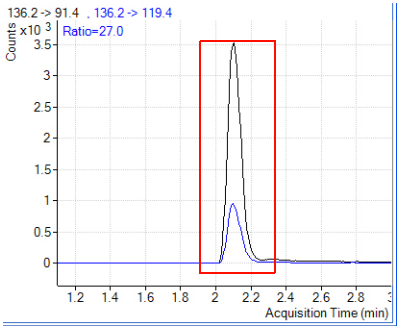
Steps	Detailed instructions	Comments
	d Mark the **Baselines** check box in the **Properties** dialog box. Click the **Apply** button and observe the peak with the baseline drawn.	

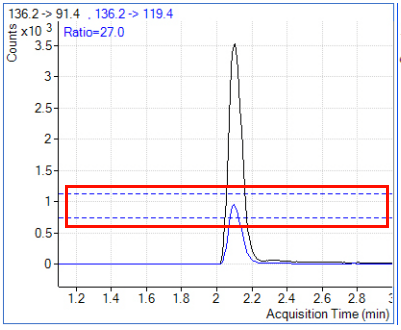
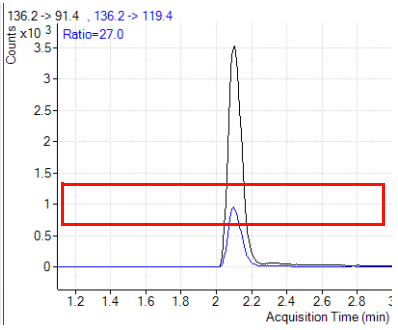
Steps	Detailed instructions	Comments
7 Inspect the calculation points for the baseline for amphetamine.	**a** Mark the **Baseline Calculation Points** check box in the **Properties** dialog box. **b** Click **Apply** and observe where the baseline starts and stops.  **c** Clear the **Baseline Calculation Points** check box in the **Properties** dialog box. **d** Click **Apply** and observe the chromatograms. **e** Compare the chromatograms with and without Baseline Calculation Points.  	

Steps	Detailed instructions	Comments
8 Display the peak labels for amphetamine. • Display those found in the figure on the next page. • Then display the original retention time peak label.	**a** Click **Peak Labels** from the **Properties** dialog box. The system displays the **Peak Label dialog** box. **b** Mark all the **Peak Labels** check boxes, and the **Display Label Names** check box, Click **OK**.	



Steps	Detailed instructions	Comments
	c Click the **Apply** button in the **Properties** dialog box. The peak labels should now match those shown in the example below. 	
	d Click **Peak Labels** in the **Properties** dialog box. The system displays the **Peak lables** dialog box. **e** Clear all the **Peak Labels** check boxes except RT (retention time). Clear the **Display Label Names** check box, and click **OK**. **f** Click **Apply** in the **Properties** dialog box and observe the change in Peak Labels.	

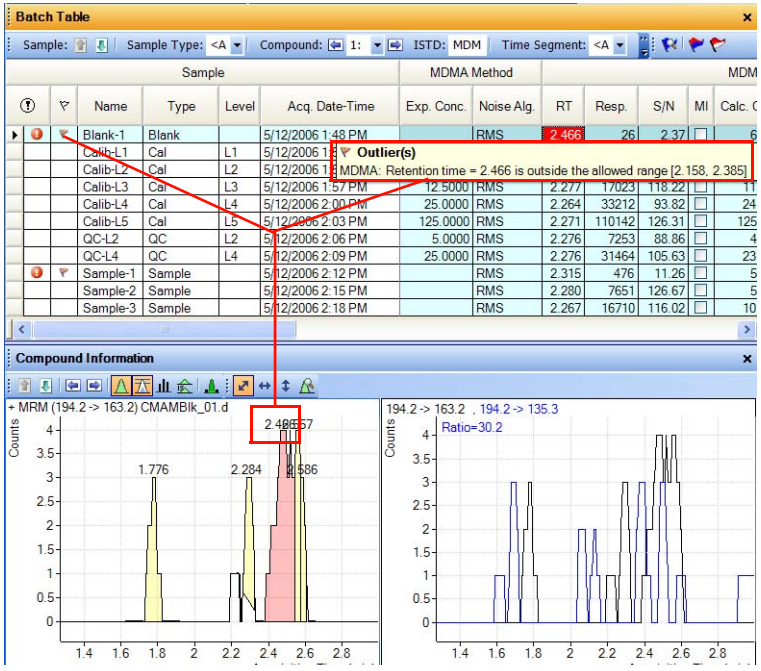
Steps	Detailed instructions	Comments
9 Display the qualifier chromatogram on the right-side before and after normalization.	**a** Mark the **Normalize** check box in the **Qualifiers** section of the **Properties** dialog box. Click **Apply** and observe that the two peaks now converge and appear as one peak.	• For B.04.01 and later revision: Notice that the default setting displays the normalized qualifier peak overlaid on the quantifier peak.
		
	b Clear the **Normalize Qualifiers** check box of the **Properties** dialog box. Click **Apply** to display the qualifier 2nd quantifier peaks again.	
		

Steps	Detailed instructions	Comments
10 View the uncertainty band.	**a** Select the type of uncertainty band you would like to display from the drop-down menu in the **Uncertainty Band** field of the **Properties** dialog box. Click **Apply** and the uncertainty band appears in the qualifier chromatogram. **b** Select **No band** from the **Uncertainty Band** drop-down menu of the **Properties** dialog box. Click **Apply** to remove the uncertainty band from the qualifier chromatogram. **c** Click **OK** to close the **Properties** dialog box. **d** Compare the qualifier chromatogram with and without the **Uncertainty band**.  	• Uncertainty band - a dashed band that shows the upper and lower boundaries for the qualifier abundance
11 Remove the **Int.** and **Int. Parms.** columns from the **Batch Table**.	**a** Click the **Restore Default Layout** button. **b** Right-click the **Meth Method** section of the **Batch Table**, and click **Add/Remove Columns**. **c** Select **Int.** and **Int. Parms.** (Compound Methods) from the right-hand list. **d** Click **Remove**, then **OK**.	

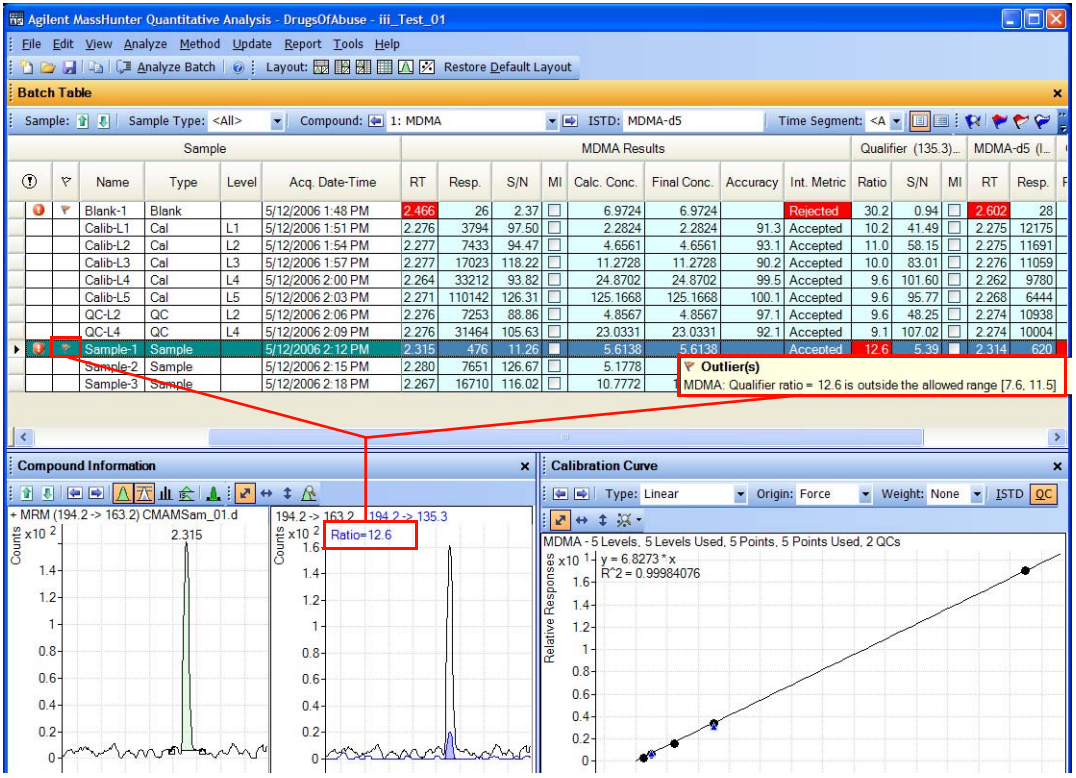
Task 3. Detect Outliers

This task shows you how to fine-tune the accuracy range for a compound and hide and show results with outlier flags.

Steps	Detailed instructions	Comments
1 View outlier information for MDMA.	**a** Click **Next Compound** in the Batch Table toolbar until the system displays the compound MDMA. **b** Select the **Blank-1** row, and point the cursor to the **RT** column, as shown in the example below.	

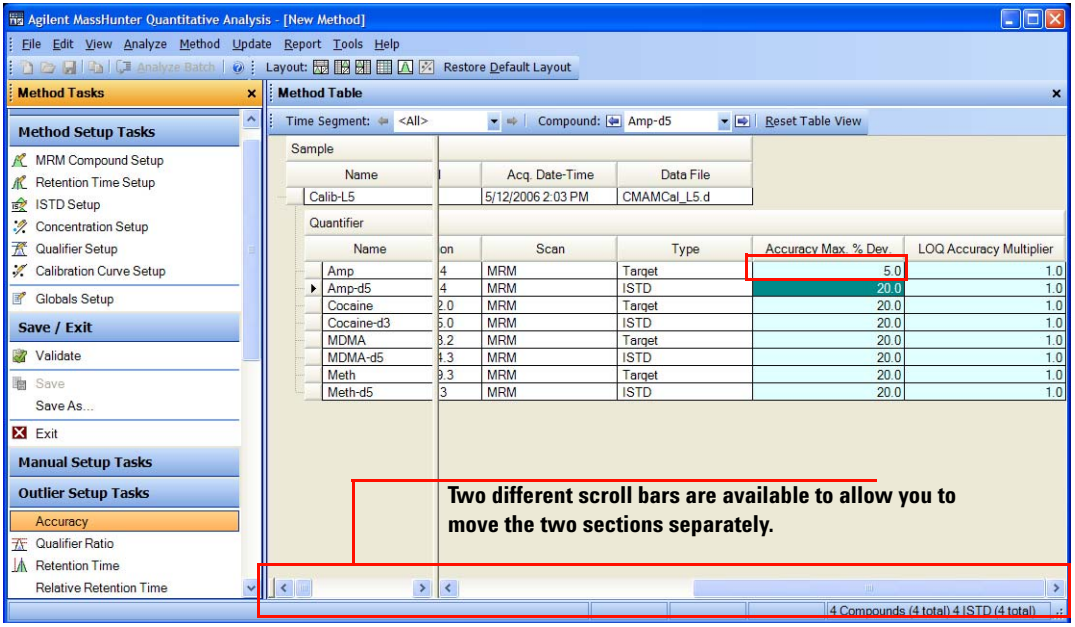


Steps	Detailed instructions	Comments
	c Examine the outlier information in the **Qualifier ... Results > Ratio** column for Sample 1, as shown in the example below.	



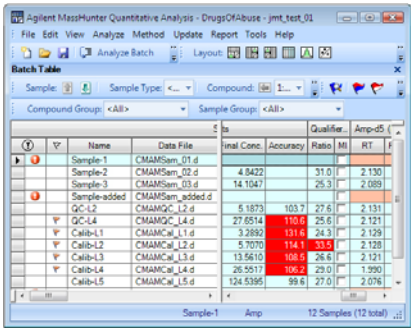
2 Change the accuracy range for amphetamine in the method, and reanalyze the batch. • Set the accuracy maximum percent deviation (**Accuracy Max % Dev**): to **5%**.	**a** Click the **Previous Compound** icon in the toolbar until the system displays the compound **Amp**. **b** Select the **Calib-L5** row in the table. **c** Click **Method > Edit** to switch to method editing mode. **d** Click **Method Tasks > Outlier Setup Tasks > Accuracy**. **e** Set the **Accuracy Max % Dev** value to **5%** for **Amp**.	You can split the **Method Table** by dragging the small rectangle to the left of the scroll bar. In the example below, the rectangle next to the bottom scroll bar was used to split the **Method Table**. The information in the two sections is exactly the same. You can use these two panes to look at two sections of the table at the same time.

Steps	Detailed instructions	Comments



f Click **Method Tasks > Save/Exit > Exit,** and click **Yes** in the confirmation prompt to exit the method and apply the method to the batch.

g Press F5 to analyze the batch. Red (high) and blue (low) outlier values now appear in the **Accuracy** column for Amp.



You can also split the **Batch Table** into two sections. By default, the **Sample** columns are locked in position and only the other columns are scrolled. If you split the table into two sections, you can determine which columns appear in each section. You need to clear the **Lock Sample Columns** menu item in the Batch Table shortcut menu if you split the **Batch Table**.

Steps	Detailed instructions	Comments
3 Using the following set of outlier flag icons  : • Check for samples with high outliers • Check for samples with both high and low outliers • Display all samples again. • Hide the outlier flags for **Accuracy** and **RT** for Amp. • Show these outlier flags again	**a** Click the **Display samples that have High outliers**  icon on the toolbar to display only samples with high outliers. **b** Click the **Display samples that have High/Low outliers**  icon on the toolbar to display only samples with low outliers. **c** Click the **Display samples that have High/Low outliers**  icon again to display all the samples. **d** Click the **Select Outliers**  icon to bring up the **Outliers** dialog box. **e** Clear the **Accuracy** and **Retention Time** check boxes, and click **OK**. **f** Click the **Select Outliers**  icon to bring up the **Outliers** dialog box. **g** Mark the **Accuracy** and **Retention Time** check boxes, and click **OK**.	• Note that to restore the **Batch Table** to view all data files, with and without outliers, simply click again on the icon you selected for filtering outliers.

Exercise 5
Generate Quantitation Reports

This exercise helps you learn how to do these tasks:

- Generate reports using a single template
- Review the reports

The **DrugsOfAbuse** batch is used in this exercise. The same tasks can be performed with Triple Quad data files, Q-TOF data files, and TOF data files.

Each exercise is presented in a table with three columns:

- Steps – Use these general instructions to proceed on your own to explore the program.
- Detailed Instructions – Use these if you need help or prefer to use a step-by-step learning process.
- Comments – Read these to learn tips and additional information about each step in the exercise.

The Advanced tab in the Reports designer provides more options in customizing your reports. For detailed information on Advanced options, see the *Online Help*.

Task 1. Generate a Report Using a Single Excel Template

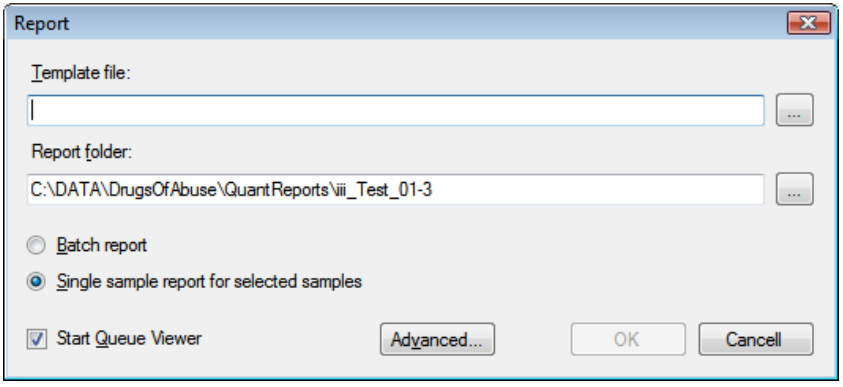
There are two main criteria that determine how reports will be created in MassHunter, they are:

- The **Report Template** you choose. (There are more than 50 pre-defined Excel templates to use. See *Online Help* for the complete list.)
- The **Report Mode** you select. (Batch versus Sample)
  - A **Batch** Report Mode creates one Excel file for the entire report.
  - A **Sample** Report Mode creates one Excel file for each sample you specified.

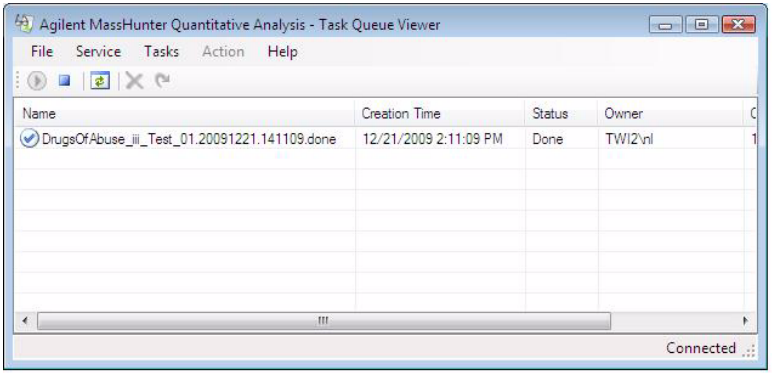
In this task, you will first generate a Single Sample Report using one Excel template, and then a full Batch Report using the same template.

Steps	Detailed instructions	Comments
1 If necessary, open the batch file ***iii*_Test_01.batch.xml**. If the batch is already open, skip to step 2.	**a** To start the Quantitative Analysis program, click the **Quantitative Analysis (QQQ)** icon on your desktop. **b** Click **Open Batch** on the toolbar to display the **Open Batch** dialog box. **c** Navigate to \ ***Your Directory*\ DrugsOfAbuse** and click ***iii*_Test_01.batch.xml.**	• You can also access the program by clicking **Programs > Agilent > MassHunter Workstation > Quantitative Analysis (QQQ)** from the Start menu. • If the default layout is not present, click **Restore Default Layout** on the toolbar before opening the batch. Restore Default Layout
2 First generate a single sample report for **Sample 1**. Highlight **Sample 1** in the Batch Table.	If you wanted to generate a report for multiple samples you would use the shift and control keys to select multiple samples from the Batch Table.	• A separate sample report will be generated for each sample highlighted in the Batch Table. In this case, we selected only one sample, so only one sample report will be generated.

Steps	Detailed instructions	Comments
3 Select a report template. • Add the template **Quantreport_ISTD_ByCompound_B_04_02.xlt.**	**a** Click **Report > Generate** from the toolbar. The system displays the **Report** dialog box. **b** Click the **browse** button next to the **Template file** field to display the **Open** dialog box. **c** Navigate to the **Letter\ISTD\ByGroup** directory then select **Quantrport_ISTD_ByCompound_B_04_02.xlt** and click **Open**. The program adds the template to the **Template file** field in the **Reports** pane.	• Note that the **B_04_02** designation corresponds to the Quantitative Analysis software release, which will change over time. Therefore, the default report file name may change correspondingly.
4 Accept the default destination directory for this report.	You may change the destination directory for saving the Excel report in the **Report Folder** text box; for example, \ ***Your Directory*** **DrugsOfAbuse****QuantReports**\ ***iii*** **_Test_03**. However, in this case, accept the default.	• The **Report folder** field allows you to specify the folder to store your report. The software defaults to a dash-numbered subdirectory with the same name as the batch located in the **QuantReports** subdirectory in your batch directory. Select the **browse** button next to the **Report folder** field to navigate to a different directory. The report is named automatically, according to your data file, with an sequentially numbered extension.



Steps	Detailed instructions	Comments
5 Select the **Single sample report for selected samples** radio button.	This option will produce a separate **sample report** for each selected sample, in this case **Sample1**.	• A single sample report will be generated based on each Excel template. In this case, we used one template, so one Sample report (one Excel file) will be created.
6 Click **OK** to generate the report. • View the status of the report generation in the **Task Queue Viewer**.	**a** Check the **Start Queue Viewer** check box if you would like to view the progress of your report as it is generating. **b** Click **OK** in the **Report** dialog box to generate the report. **c** Watch the progress of the report in the **Status** column. Once the report is complete you will be prompted, and the status column will indicate that the report is done.	• All reports generated are displayed in the viewer. The most recent display at the top of the list.



7 To view or print the report, open it in Excel.	**a** Highlight the report you want to view. **b** Select **Action** from the toolbar in the **Task Queue Viewer** dialog box. A drop-down menu is displayed with the most recent reports generated. **c** Double-click your report file to open the Excel spreadsheet.	• Reports are viewed or printed from Excel. • Alternatively, you may open the spreadsheet by selecting the file in Windows Explorer.

Steps	Detailed instructions	Comments
8 Now generate a batch report using this same template. Return to the Batch Table (described in step 1) .	Exit the Excel spreadsheet and return to ***iii_Test_01.batch.xml.***	
9 Repeat steps 3, and 4 as described above.		
10 Select the **Batch report** radio button.	This will produce a single batch report (a single Excel file) containing *everything in the selected batch*.	
11 Repeat steps 6 and 7 to generate and view the report.		

Reference

Ten Main Capabilities

Quantitative Analysis includes ten capabilities that help you integrate, quantitate, and review your data more easily and powerfully:

Batch-at-a-Glance: Batch Table Setup

- New batch — Creates a batch table in which you can operate on samples and compounds from a single view
- Analyze — Recreates the calibration curve and requantitates all samples using the method that is currently open
- Quantitate — Applies the existing calibration curve to the current batch, sample, or compound

  The granularity of applying quantitation allows you to quickly manipulate a particular signal.
- Integrate — Integrates signals to the current batch, sample, or compound

Method Editor

- MRM Setup — Presents a quantitation method in simple stepwise fashion
- Create method from acquired MRM data — Creates a quantitation method automatically from the acquisition method after requiring only the assignment of ISTD relationship and concentrations
- Create a method manually using the graphics in the Sample Information window
- Group by time segment — Organizes methods by compounds in ordered time segments
- Validate — Ensures that a quantitation method meets rigorous criteria
- Isotopic dilution — Supports adjustments from (Rx, Ry) Colby constant calculations

Calibration

- CurveFit assistant — Calculates all combinations of curves; picks disabled points; and presents results with an equation that is sortable by confidence band and custom filterable by R^2, standard error, and max % residual
- Dilution assistant — Calculates and creates calibration levels based on a default or specified serial dilution scheme

- Copy Cal levels — Copies calibration levels from one compound to other compounds
- Disable Cal points — Disables calibration points based on level, or individual compounds in tables, or interactively through graphs
- Curve fits — Supports curves by:
  - Type: Linear, Quadratic, First order ln, Second order ln, Average of Response Factors
  - Origin: Ignore, Include, Force, Blank Offset
  - Weight: None, $1/x$, $1/x^2$, $1/y$, $1/y^2$, Log, $1/SD^2$
- Replace curve — Creates calibration curves from existing calibration samples
- Average replicates — Averages in new replicates into existing calibration curves by compounds
- Import levels — Imports calibration levels and concentrations from a file
- Scale graphs — Provides graphs with the capability to be autoscalable by X, Y, X-log, and Y-log; and intelligent zooming to fit specified levels

Integrator

- MS-MS integrator — Provides a parameter-free integrator at all levels of signals that reduces manual integration efforts
- Integrator metrics — Generates metrics that characterize the signal's integration to accept, inspect, or reject the integration
- Signal-to-noise — Calculates signal-to-noise for peaks
- Graphics — Shows superior interaction with the graphing of a compound and the display of peak information

Batch-at-a-Glance: Results

- Navigation — Moves (previous, next, direct) between samples, compounds, time segments, and compound groups
- Compound views — Switches between the details of the current compound or the summaries of multiple compounds
- Batch Table views — Enables flat-table layouts or the capability to drill down to vertically or horizontally nested tables for details
- Window layout — Reorganizes the screen to its defaults, or saves or loads custom-window layouts

- Float pane – Floats any pane onto another monitor to enable dual-monitor presentations
- Export Table – Exports Batch-at-a-Glance tables directly to Excel files
- Export Graphics – Exports any graphic to a customized size in multiple formats
- Copy/Paste – Copies or pastes any graphic directly into Microsoft Office applications such as Word, PowerPoint, Excel, etc.
- Print/Preview – Prints or previews screen content in WYSIWYG format (what-you-see-is-what-you-get)
- AutoReview – Displays each sample automatically and interactively, allowing you to stop at any time for closer inspection
- Filter – Displays any combination of sample types
- Sort – Sorts any column that appears in a table
- Columns – Enables you to add, remove, reorder, save, load, restore, or reset columns

Compounds-at-a-Glance: Results

- Print/Preview – Prints or previews compound chromatagrams.
- Copy/Copy Page – Copies selected compound chromatograms, or all compound chromatograms on the screen into Microsoft Office applications such as Word, PowerPoint, Excel, etc.
- Edit Compound Chromatograms – Manually integrate the data, or select zero-peak compounds.
- Views – Displays chromatogram details such as baselines, filled peaks.
- Adjust Axes – Link/Unlink X or Y axes, autoscale to fit the panes, fit to peaks or fit to calibration levels.
- Layout – Organize rows by compounds or samples, select chromatogram overlays, review sample by sample or compound by compound, set display options.
- Highlight – Compounds with outliers

Outlier Detection

- Manage – Sets up and selects specific outliers that can be detected and individually controlled

- Highlight — Highlights outlier values (high = red, low = blue) in the results table
- Filters — Lets you display selected types of filters
- Outliers — Supports specific types of data for outlier detection
- Quantitation message — Warns you of samples that encountered serious problems during quantitation

Report

- Generate — Generates graphics and report results for importing and formatting for Excel XML
- Custom — Lets you customize the Excel template

Update

- Update/Average RT — Updates or averages compound's retention times
- Update Qualifier Ratios — Updates qualifier ratios based on the compound's current sample
- Update Mass Assignments — Updates mass assignments based on compounds current sample

Qualitative

- Sample Information — lets you display the chromatogram and extracted spectra for the current sample
- Chromatogram/Spectrum — Provides significant features that can be used to explore spectra for different types of signals

Quantitative Methods

The Method Editor lets you create a new quantitation method from an MRM acquisition data file (Figure 13), from SIM data, from an acquired Scan data file, or manually.

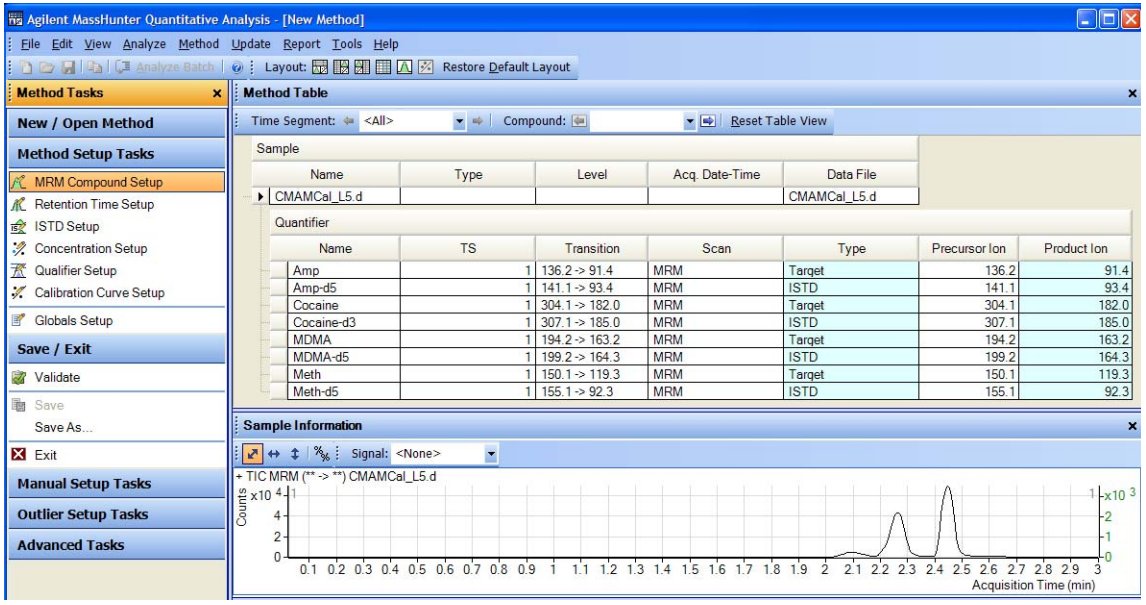


Figure 13 Quantitative view – Method Editor

A file selected from the Batch Table is used as a reference for developing the method settings. These settings are then used to generate the calibration curve and quantitate the standards, QCs, and samples.

Parameter-Free Integrator

What is the parameter-free integrator?

Agilent has developed a new peak integrator algorithm that works especially well for MS/MS data. The parameter-free integrator presents these advantages:

- Handles low-level noisy data by setting a peak's starting and ending points statistically
- Adjusts the threshold automatically
- Eliminates the need for manually reintegrating peaks for low-level MRM signals
- Identifies those peaks that appear reliable and those that should be discarded

Example of integration results

Figure 14 shows data at two extremes.

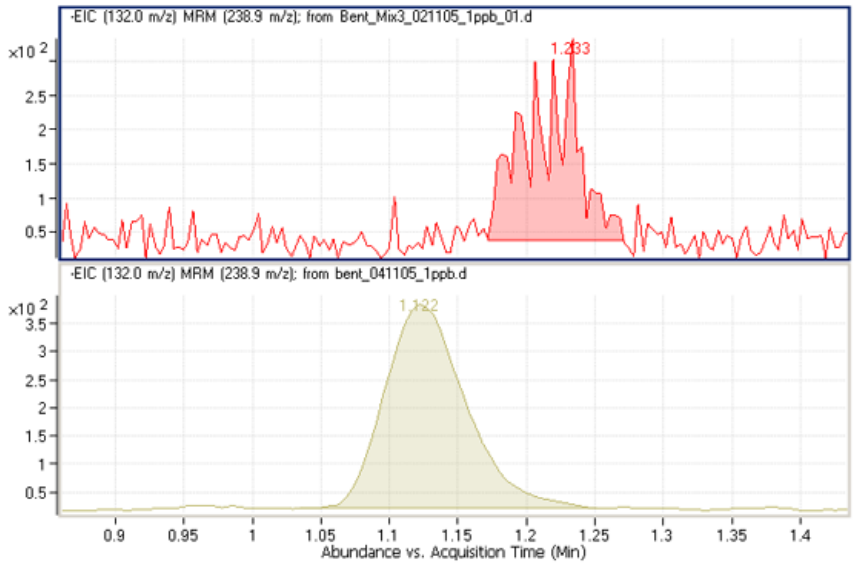


Figure 14 Parameter-free integrator – Data at two extremes

The lower chromatographic peak could be easily integrated since it is a nice Gaussian-shaped peak, but it would be difficult to define the baseline of the upper peak. In fact, many integrator algorithms might interpret these results as multiple peaks.

However, Agilent's new algorithm had no trouble defining the baseline and recognized this as a single peak. In fact, the new integrator algorithm would integrate this as a single peak even if the baseline were rising, instead of being flat, as shown.

Batch-at-a-Glance: Results

The integration results obtained from the analysis of amphetamine (Amp) are shown in Figure 15. This is a flat view of the **Batch Table**, **Compound Information**, and **Calibration Curve**.

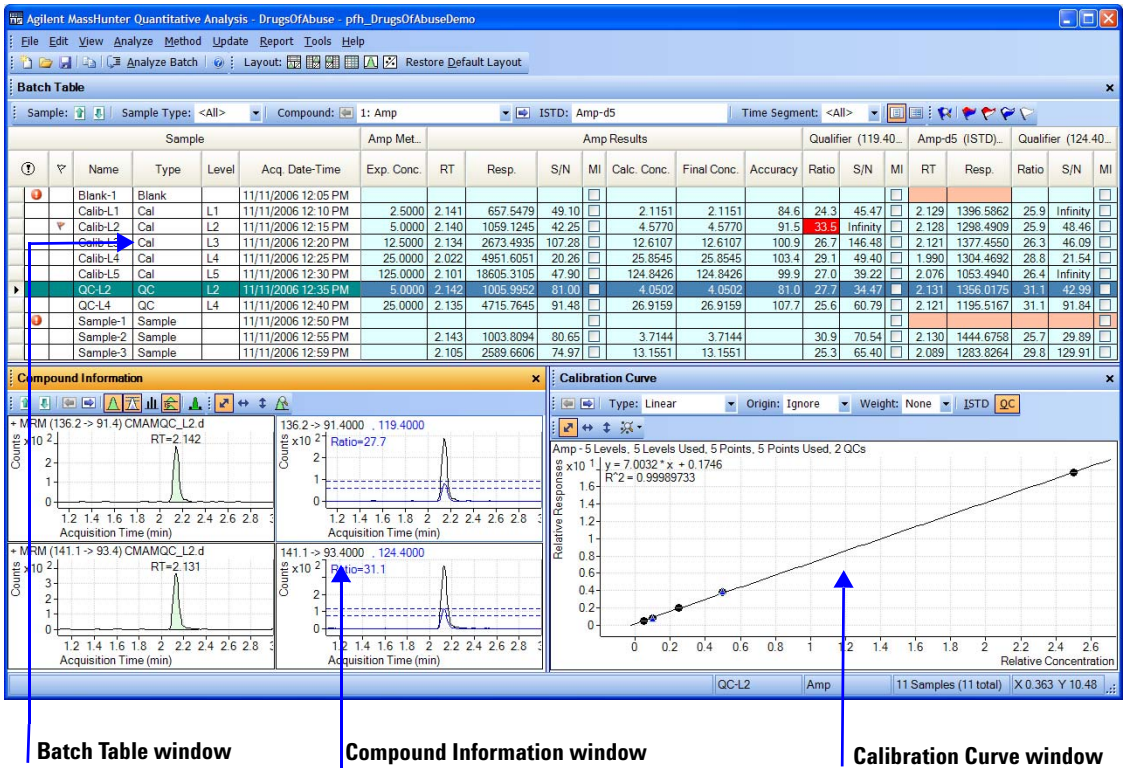


Figure 15 Amp results

- The **Batch Table** shows the integration results from applying the quantitation method to each data file. Colored highlights correspond to results that are lower (blue) or higher (red) than expected.
- The **Compound Information** window at the lower left displays the integrated chromatographic peaks.
- The **Calibration Curve** is shown at the lower right.

Compounds-at-a-Glance

The Compounds-at-a-Glance view shows specific compounds detected in each sample, as shown in Figure 16. This feature allows you to view the compound chromatograms and arrange them for easy data analysis. It is especially useful for food safety labs that look for compound trends within batches of samples.

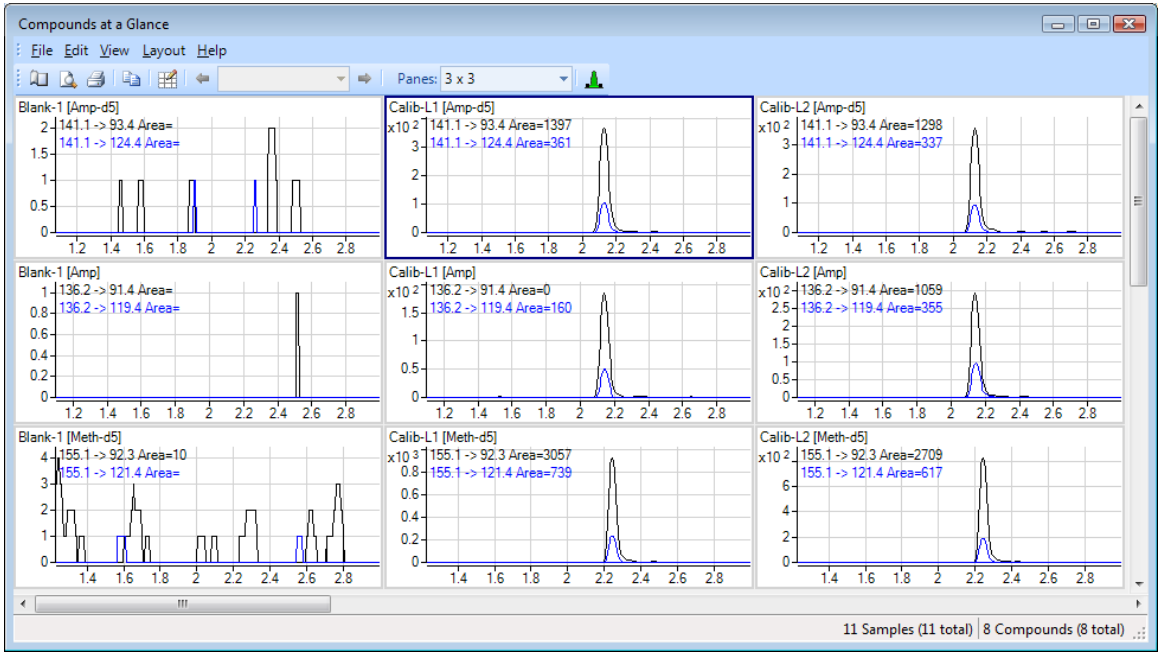


Figure 16 Compounds-at-a-Glance in Quantitative Analysis

The setup feature in the Compounds-at-a-Glance allows you to select the compounds and samples you would like included in the view. As shown in Figure 17 the different tabs at the top of the **Setup Graphics** box provide different options for selecting and arranging the chromatograms.

- The **Samples** tab lists all the samples included in the batch, and gives options for selecting all samples or specific samples.
- The **Compounds** tab lists the compounds detected in the batch. It allows you to choose the compounds you would like to view.

- The **Organize** tab allows you to specify the arrangement of the chromatograms, according to sample and compound. It provides overlay options for compounds, samples, and outliers. The tab gives choices for adjusting the chromatograms, such as displaying baselines or fill peaks to best illustrate compound detection trends.
- The **Outlier** tab provides options for showing outliers in the data.

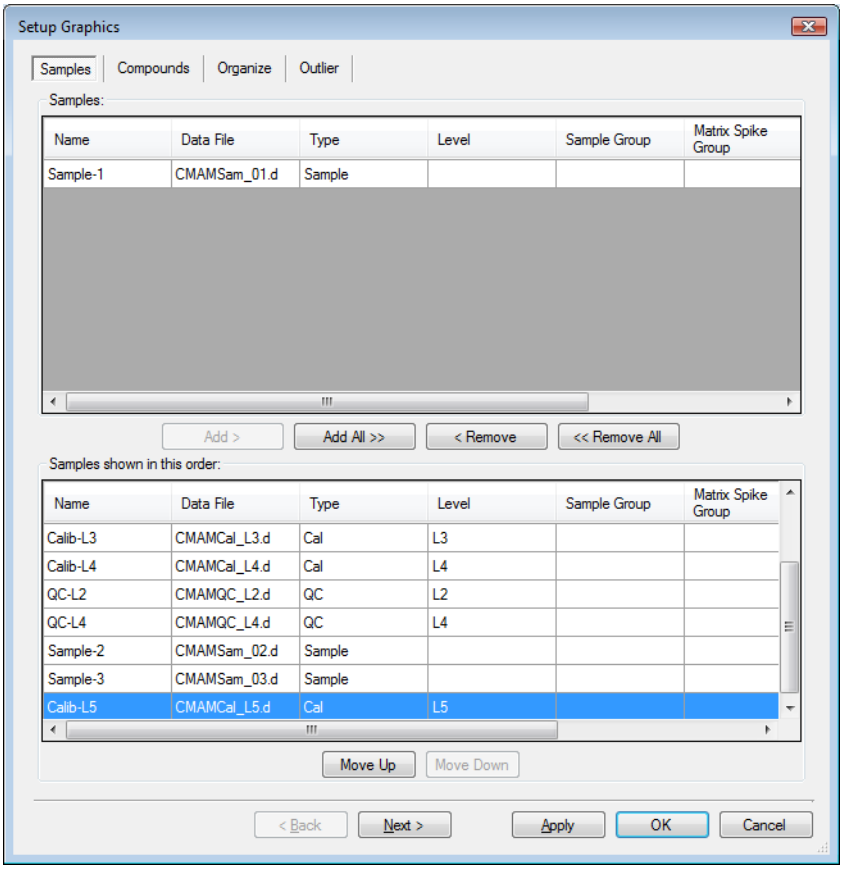


Figure 17 Setup options for Compounds-at-a-Glance

Compound Confirmation

The format shown in Figure 18 can be of value to certified drug-testing laboratories. It shows two sets of plots that can be obtained from a THC analysis.

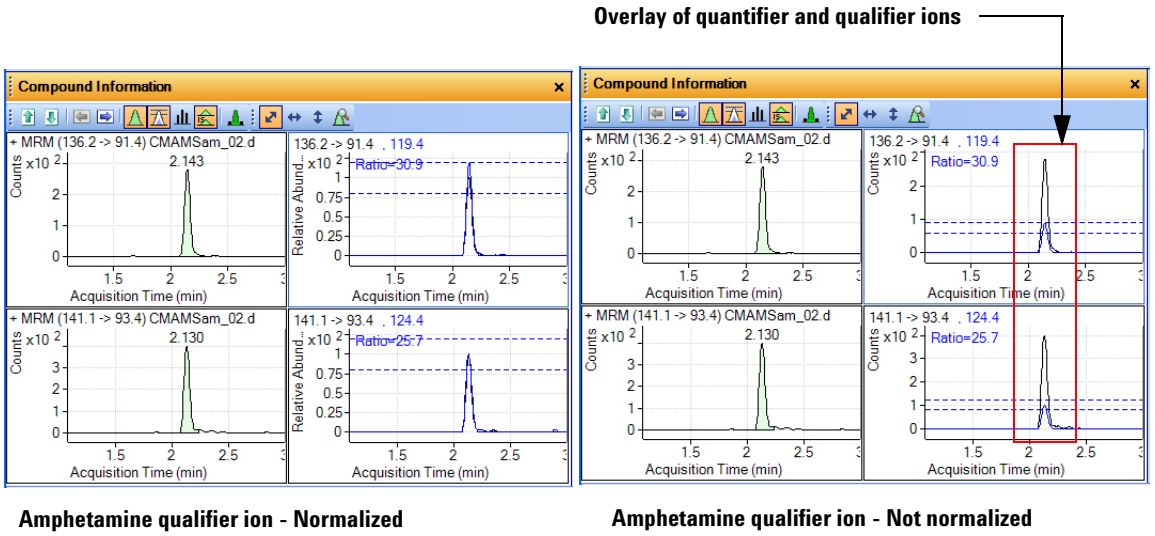


Figure 18 Amp in Quantitative Analysis

Two product ions must be acquired for confirmation: a quantifier ion and a qualifier ion. Typically, the quantifier ion that is used for quantitation is the most abundant of the two product ions.

To be able to confirm the presence of Amphetamine, the qualifier ion peak area must be at least a certain percentage of the quantifier ion, a number that is set in the quantitation method. In this example, 26.5% is used with a window of ± 20%. This means that the area of the qualifier ion must be in the range of 21.2 to 31.8% of the quantifier ion for the analyte Amp. The qualifier for the ISTD, or Amp-d5, also has a specific range that it must be in.

From the figure on the left, whether or not the qualifier ion falls within the accepted window is not easily determined because the size of the qualifier peak is normalized by a factor of 1/0.265. In the figure on the right, the acceptance window is centered at 26.5% of the quantifier ion peak and the

qualifier ion is drawn not normalized, or on the same scale as the quantifier. If the ion is not within the required acceptance window, then it is shaded blue, but is still transparent so as not to hide the quantifier ion. This makes it easier to confirm the presence of compounds visually.

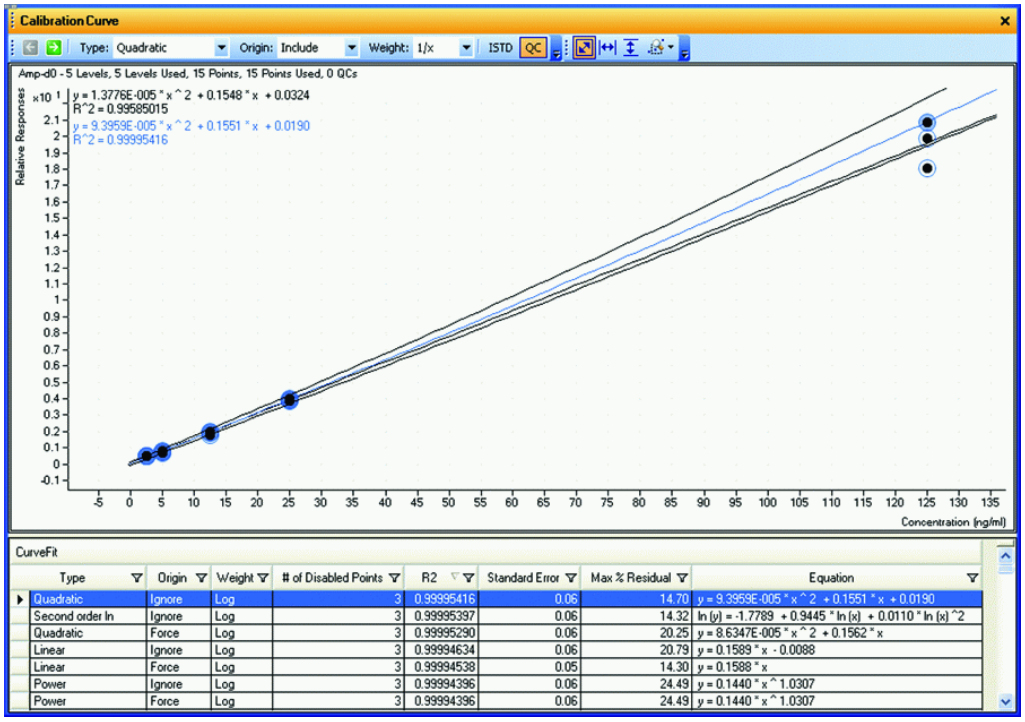
Compound Calibration

The Quantitative Analysis program contains several tools to help calibrate and quantitate compounds:

- CurveFit Assistant
- Cursor Pointer for Data Point Information
- Data Point Zooming

CurveFit Assistant

The CurveFit Assistant provides an analytical view of evaluating the possible curve fits (Figure 19).



Type	Origin	Weight	# of Disabled Points	R2	Standard Error	Max % Residual	Equation
Quadratic	Ignore	Log	3	0.99995416	0.06	14.70	y = 9.3959E-005 * x ^ 2 + 0.1551 * x + 0.0190
Second order ln	Ignore	Log	3	0.99995397	0.06	14.32	ln (y) = -1.7789 + 0.9445 * ln (x) + 0.0110 * ln (x) ^2
Quadratic	Force	Log	3	0.99995290	0.06	20.25	y = 8.6347E-005 * x ^ 2 + 0.1562 * x
Linear	Ignore	Log	3	0.99994634	0.06	20.79	y = 0.1589 * x - 0.0088
Linear	Force	Log	3	0.99994538	0.05	14.30	y = 0.1588 * x
Power	Ignore	Log	3	0.99994396	0.06	24.49	y = 0.1440 * x ^ 1.0307
Power	Force	Log	3	0.99994396	0.06	24.49	y = 0.1440 * x ^ 1.0307

Figure 19 CurveFit Assistant

Note that the black line drawn through the data points uses Quadratic as the Fit, 1/x as the Weight, and Include as the Origin as shown at the top. Many other combinations of the curve settings are listed below the calibration curve, with the selected one highlighted in blue. The highlighted settings are also plotted in blue in the curve window.

You can find the best curve fit, for example, one that corresponds to the highest R^2 value, by ordering all of the possible results from the best to the worse R^2 values and then deciding how many data points to consider as being outliers.

For example, the first set of parameters in the list corresponds to a Linear Fit, Ignore Origin, and Equal Weight. The corresponding R^2 value is 0.9998001477, which is very good. The corresponding curve can be plotted by simply clicking this entry in the table.

Using these settings, data can be requantitated. Eliminating outliers is common as a standard operating procedure (SOP) in some laboratories.

Data point information

Overlapping data points are not unusual in a calibration curve, especially with triple quad MS data, where %RSD values are quite low (Figure 20). To help distinguish the data points from one another, the cursor can be moved over the data points to obtain more information about them.

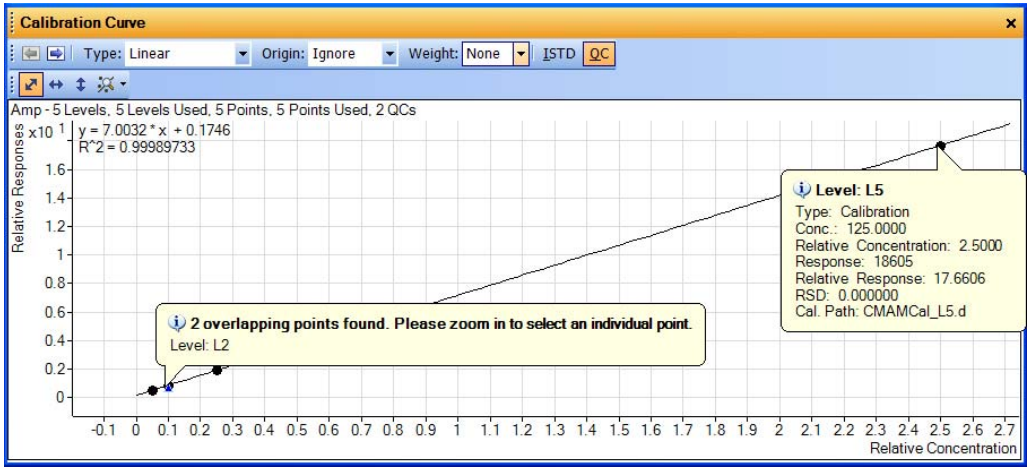


Figure 20 Amp results: Calibration data point information

This figure shows two examples of this type of information. The first example shows that the data points overlap and advised you to zoom in to see them separately. The second example shows information on the data point itself.

Data point zooming

You can zoom in on overlapping data points to see individual data points not visible in the visual presentation.

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In This Book

The Familiarization Guide presents exercises to help you use the Quantitative Analysis program. In this guide you learn:

- How to set up and quantitate a batch of Agilent Triple Quad LC/MS and GC/MS data files
- How to set up and quantitate a batch of Agilent Q-TOF LC/MS data files
- How to inspect your quantitation results and spot irregularities
- How to improve result accuracy
- How to generate and review quantitation reports

Fourth Edition, April 2010
G3335-90061

Agilent Technologies